Dilution of Semisolid Preparations

Studies on the parameters affecting hydrocortisone release and permeation through excised human stratum corneum with emphasis on the influence of dilution

Verdünnung von Halbfesten Systemen

Beitrag zu den Einflußparametern auf die Hydrocortison-Freisetzung und Hydrocortison-Permeation durch exzidiertes humanes Stratum corneum unter besonderer Berücksichtigung des Verdünnungseinflusses

Von der Gemeinsamen Naturwissenschaftlichen Fakultät
der Technischen Universität Carolo-Wilhelmina
zu Braunschweig
zur Erlangung des Grades einer
Doktorin der Naturwissenschaften
(Dr. rer. nat.)

genehmigte
Dissertation

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eingereicht am:	20.11.2000
mündliche Prüfung (Disputation) am:	16.03.2001
	2001

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The work described here was done under the supervision of Prof. Dr. C. C. Müller Goymann at the Institute “Pharmazeutische Technologie, Technische Universität Carolo-Wilhelmina”, Braunschweig.

I would like to express my great gratitude to

Prof. Dr. C. C. Müller Goymann

for the kind supervision of my thesis. Prof. Dr. C. C. Müller Goymann has been very helpful and her encouragement, support, valuable guidance and criticism were the corner stone for the achievement of this work.

My sincere thanks are also for Prof. Dr. G. Lee for the second evaluation report.

It gives me a great pleasure to express my thanks to all co-workers of the institute for their readiness to help, the fruitful discussions which contributed to the success of the work and for the wonderful working atmosphere which has positively reflected on the smooth running of the work.

The skin samples were kindly obtained from Dr. Flory “Hollwede Krankenhaus Braunschweig” to whom I express my gratitude.

I also want to express my thanks to Knoll AG, Ludwigshafen for providing me the placebo of Soventol creme.

I am greatly indepted to my husband for his encouragement, help and useful comments during the whole period of the work.

The following parts of the thesis have been published after approval of the Faculty of Science as:

Articles

H. Refai and C. C. Müller-Goymann, Larvated incompatibilities of hydrocortisone cream preparations upon dilution with different cream bases. Pharmazie 54, 1999, 754-758

C.C. Müller Goymann and H. Refai, Tücken im Apothekenlabor. Pharm.Ztg. (37) 145, 2000, 11-16

Posters

H. Refai and C. C. Müller-Goymann, Larvated incompatibilities of hydrocortisone cream preparations upon dilution with different cream bases. 13. Fachgruppentagung (Expert Meeting) of the German Pharmaceutical Society (DPhG), Freiburg, 1999

H. Refai and C. C. Müller-Goymann, Effect of diluting hydrocortisone cream preparation with different cream bases on permeation through excised human stratum corneum. Annual Congress of the German Pharmaceutical Society (DPhG), Frankfurt (M), 1999

H. Refai and C. C. Müller-Goymann, The influence of changing the rheological properties of hydrocortisone cream preparation upon dilution with different cream bases on drug release. 3rd World Meeting on Pharmaceutics, Biopharmaceutics, Pharmaceutical Technology, Berlin, 2000

H. Refai and C. C. Müller-Goymann, Influence of diluting a cream preparation containing an enhancer on permeation through excised human stratum corneum. Annual Congress of the German Pharmaceutical Society (DPhG), Münster, 2000

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1. Introduction

The dilution of commercially available topical corticosteroid formulations is a common practice done in many countries in response to physicians' requests. The intuitive expectation is that dilution would reduce the activity of the corticosteroid formulations to meet the needs of the patients and at the same time enable the physician to restrict prescribing to well known corticosteroid molecules. However, predicting the extent to which activity is reduced when a topical corticosteroid formulation is diluted is no simple task, besides this practice is associated with a number of dangers. In particular, there is a risk of accelerating chemical and physical decomposition, facilitating microbial contamination and interfering with the biopharmaceutical profiles of the formulations.

The biopharmaceutical incompatibilities, which may arise from the inappropriate selection of the vehicle used for dilution, are of special importance as the sophisticated nature of the commercially available bases may imply that any changes in concentration or components of the base may affect the rate of release of the corticosteroid from the final preparation. It would therefore appear that extemporaneous dilution of proprietary topical corticosteroid formulations could result in a disturbance of the equilibrium of base components necessary for optimum release of active ingredients.

The aim of this study was to investigate to what extent the drug release from topical semisolid preparations as well as drug permeation through skin are influenced by dilution. For this purpose water-containig hydrophilic ointment DAB 1998 (WHS) with 1% hydrocortisone was chosen as a model cream base. It was diluted with various hydrophilic and lipophilic vehicles with and without water content all selected from the German Pharmacopoeia, DAB 1998. The following aspects were investigated:

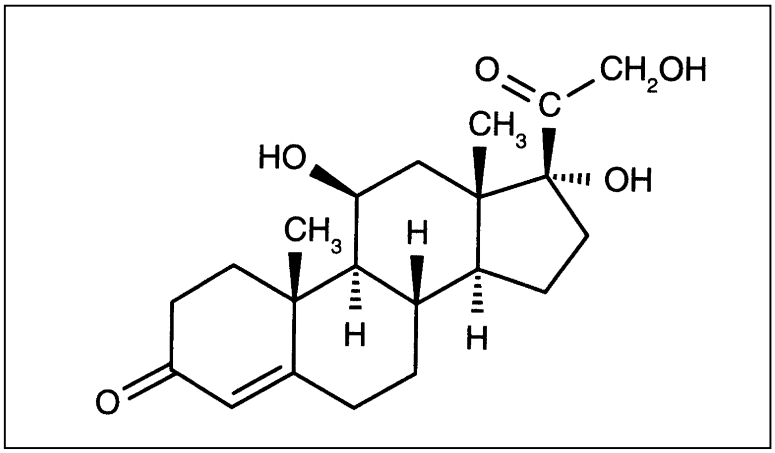
a) The influence of base type used for dilution and degree of dilution on the release of hydrocortisone from topical semisolid preparations.
b) Factors affecting drug release such as drug solubility in the base, water content and viscosity in order to interpret the different liberation profiles.

c) Effect of base type and dilution on drug permeation through excised human stratum corneum and the correlation between liberation and permeation studies.
d) The interactions between the cream bases used for permeation with the structure of stratum corneum and the subsequent influence on the permeability of stratum corneum.

2. Theoretical part

2.1. Hydrocortisone

Hydrocortisone was introduced 1952 by Sulzberger and Witten as the first topically applied corticosteroid which represented a great advance on previously available therapies (Structure, Fig. 2.1.)



However, the halogenation of corticosteroids in the positions C-6 and C-9, first introduced by triamcinolone acetonide began the revolution which cumulated in the appearance of the very potent agents now available.
Hydrocortisone is classified as a low potency corticosteroid with comparable efficacy to dexamethasone and flumethalone (Cornell and Stoughton, 1984).
Corticosteroids posses antiinflammatory activity, immunosuppressive properties and antiproliferative actions (Miller and Monro, 1980) being very useful in the local treatment of eczema, dermatitis, pruritis, insect bites and psoriasis.

Antiinflammatory effects result from decreased formation, release and activity of the mediators of inflammation (e.g. kinines, histamine, prostaglandins and leukotriens) which reduce the initial manifestations of the inflammatory process. Moreover, corticosteroids inhibit cell migration to the area of injury and also reverse the dilation and increased vessel permeability in the area resulting in decreased access of cells

to the sites of injury. This vasoconstrictive action decreases serum extravasation, swelling and discomfort (Gilman et al., 1990).
Additional research demonstrated that glucorticoids also induce the antiinflammatory protein, lipocortin. This protein inhibits the enzyme phopholipase A2 which inhibits the synthesis of prostaglandins and lipoxygenase products (Schwiebert et al., 1996).

Immunosuppressive properties decrease the response to delayed and immediate hypersensitivity reactions. This results from inhibition of the toxic effect of antigen-antibody complexes that precipitate in vessel walls creating cutaneous allergic vasculitis. Glucorticoids also inhibit the action of lymphokines target cells and macrophages which together produce allergic contact dermatitis reactions. Additionally, the access of T lymphocytes and macrophages to target cells may be prevented by corticosteroids (Gilman et al., 1990).
Antiproliferative effects reduce hyperplastic tissue characteristics of psoriasis.

Topical hydrocortisone is ineffective unless absorbed through the skin. Therefore, regional variation in skin thickness and the presence of hair follicles may influence steroid absorption. Areas with increased permeability (scrotum, eye lids, ears, scalp and face) may respond better to topical hydrocortisone application than those areas with thicker stratum corneum (palms and soles). Factors that may facilitate steroid absorption are hydration, humidity, occlusive wrapping and damaged skin (Generali, 1985).
Ointments, for their soothing effect, are more suitable for the treatment of dry, scaly areas as well as thick and lichenified lesions. Creams and solutions are preferred for acute and subacute dermatoses, they may be used on moist skin areas (Drake et al., 1996). Hairy areas, particularly the scalp, are best treated with aerosols and lotions (Generali, 1985).

The potency of topical corticosteroids is determined mainly by the McKenzie-Stoughton vasoconstrictor assay (McKenzie and Stoughton, 1962). The vasoconstriction caused by topical corticosteroids was taken as the extent of absorption of corticosteroids in the skin since the degree of vasoconstriction, recognizable on the blanching effect, usually correlates well with the extent of clinical efficacy.

Enthusiasm for the highly effective topical corticosteroids was at its peak during the 1960s and 1970s. However, the appearance of the very serious adverse effects has created confusion and prejudice against all steroid-containing preparations which are still of considerable concern. The most common adverse effects of topical corticsteroids are:

- Atrophy
- Impaired wound healing
- Acneiform eruptions
- Hypopigmentation
- Photosensitivity
- Superinfectious-skin
- Contact dermatitis
- Systemic absorption with systemic adverse effects (Cushing-Syndrom, adrenal suppresion, growth retardation and many others)

In recent years much care has been taken to re-establish the image and certainly much effort has been done to improve the understanding of pharmacological and clinical aspects of topical corticosteroids (Maibach and Surber, 1992).

2.2. Semisolid dosage forms

Dermatological preparations are classified into liquid systems including emulsions, solutions and suspensions, solid systems (powders) and semisolid formulations.
Semisolid preparations are dosage forms characterized by being spreadable in a temperature range between room temperature and skin temperature. They are applied on the skin as well as on mucous membranes (Junginger, 1992). Semisolid preparations are considered to be colloidal systems possessing a gel structure. Münzel (1953) defined semisolid dosage forms generally as plastic gels for cutaneous application. The plastic behaviour of these systems is characterized by the existence of a yield stress after which the system begins to flow.

Ointments are water-free semisolid preparations. They are classified according to DAB 10 into three general groups:

(1) hydrocarbon bases (hydrophobic ointments), (2) absorption bases, (3) water-soluble bases (hydrophilic ointments).

Hydrocarbon bases are lipophilic in nature containing no polar components. Aqueous preparations may be only mechanically incorporated with difficulty in small amounts e.g. petrolatum, triglycerides and waxes*. Absorption bases* contain in addition to the lipophilic components also emulsifiers, which could be either w/o-emulsifiers e.g in. wool fat ointment or o/w-emulsifiers e.g. in hydrophilic ointment permitting thereby the incorporation of water resulting in the formation of w/o creams or o/w creams, respectively. *Water soluble bases* are composed of components completely miscible with water. They are characterized by the absence of any oleagenous materials e.g. propylene glycol ointment.

Creams are ointments in which an aqueous phase is incorporated. In the classic o/w creams the water is immobilized in the cream by either of two ways. On the one hand water could be bound mechanically through capillary forces and supportive gel structure. On the other hand, water could be fixed inside the hydrophilic gel phase through interfacial potential (Junginger, 1984). In contrast, in classic w/o creams e.g. water-containing wool fat ointment the aqueous phase is incorporated in the form of droplets inside the base similar to emulsions (Junginger, 1992). Amphiphilic ointments are considered to be a transition between o/w and w/o creams.

Gels are one-phase semisolid preparations consisting mainly of a liquid fixed by a gel building substance. Gels are also differentiated into hydrophilic and lipophilic gels according to the type of liquid used (Bauer et al., 1997).

Pastes are also considered as semisolid dosage forms defined as highly concentrated suspensions (Bauer et al., 1997).

The selection of the appropriate base depends greatly on the state of disease and the desired effect. A cooling, drying and antiinflammatory effect is suitable for acute and subacute cases, therefore creams and lotions are to be utilized (Drake et al., 1996). In contrast, occlusion achieved by the application of lipophilic bases enhances

the moisturizing effect of the skin through inhibition of evaporation of sweat and thereby promotes drug absorption. Lipophilic bases are therefore suitable for chronic cases. The selection of the base also depends on the skin type. Hydrophilic bases suite seborrhoic skin while lipophilic vehicles are considered to be compatible with sebostatic skin (Thoma, 1983).

2.3. Viscoelasticity

When a probe is stressed to above its yield stress it undergoes deformation. After removal of the stress the ideal viscous bodies retain the deformation, whereas the ideal elastic bodies regain their original state instantly and completely as before deformation. In these bodies the deformation energy is saved and reused afterwards for the recovery (Memory-Effect). A state between ideal viscous and ideal elastic is represented by viscoelastic bodies. They are characterized by possessing both elastic and viscous properties. After removal of the stress they are almost completely recovered but it needs longer time.
Viscoelastic bodies are characterized either by creep experiments or by oscillatory tests (Davis, 1974). In creep experiments the strain γ is recorded as a function of time for a given stress τ value. For elastic systems the deformation returns to zero after removal of the stress, whereby viscous systems remain at their maximum strain value. Viscoelastic systems show only a back-deformation of the elastic part. Viscoelastic systems react as elastic bodies when exposed to stress for short time intervals, whereas the viscous properties appear only if the probe is exposed to stress for longer time.

In oscillatory tests the system is exposed to oscillating (swinging) constant stress. At low stress values the structure of the system remains intact i.e. all parameters which characterize the system are constant. If the system is overstressed, the structure will be disturbed and the parameters drastically change.

The range of stress values in which the structure of the system is undisturbed is called viscoelastic range. This range varies from one system to the other and must be determined prior to the actual oscillatory test.
In oscillatory measurements the system is characterized by the following parameters:

Elastic modulus (storage modulus) G'
It represents the quantity of energy reversibly stored in the system, therefore it characterizes the elastic behaviour of the probe.

Viscous modulus (loss modulus) G"
It represents the quantity of irreversibly lost energy, therefore it characterizes the viscous behaviour of the probe.

*Complex modulus G** = G' + iG"

Phase angle δ
It is the phase angle by which the stress wave is shifted with respect to the strain wave.

- for ideal elastic bodies γ and τ are in phase i.e. δ is 0°
- for ideal viscous bodies there is a phase angle shift δ of 90°
- viscoelastic bodies show a phase angle shift of $0° < \delta < 90°$, therefore systems having a phase angle $\delta < 45°$ indicate exceeding elastic properties, whereas $\delta > 45°$ indicate exceeding viscous behaviour.
- tan δ (loss factor) = G´´/G´, indicating the ratio between the amount of dissipated and the stored energy and thus between viscous and elastic portions of the sample

Complex viscostity η^*
It is a measure of the viscosity of the viscous as well as the elastic parts of the probe.

2.4. In vitro release of drugs from topical products

In vitro release tests serve primarily as a quality control tool to ensure batch-to-batch uniformity and screen experimental formulations during product development. Neither universal release testing procedure nor universal test conditions exist. Rather, the release test must be tailored to a formulation i.e. suitable test conditions can usually be developed.

Generally, in the release test a layer of the semisolid dosage form is placed in contact with a reservoir and diffusion of drug out of the semisolid and into the medium of reservoir is followed. In most instances diffusive communications between the delivery system and the reservoir is through a membrane to keep the product and the receptor medium physicochemically distinct. According to suggestions of FDA, membranes are selected for use which: a) are commercially available (the practical way to assure reproducible membrane properties over time), b) have little capacity to bind a drug, c) have little tendency to interact with the releasing medium and d) offer the least possible diffusional resistance (Flyn et al., 1999).

The diffusion coefficient is taken as a parameter to describe drug release. It can be calculated from the Higuchi equations (Higuchi, 1967) but one should distinguish between “solution systems” (Eq. 2.1.), in which the drug is completely dissolved in the semisolid system and “suspension systems” (Eq. 2.2.), in which the drug is rather suspended in the base.

Release from solution systems

$$Q = 2 * A * C_0 * \sqrt{\frac{D_s * t}{\pi}} \qquad \text{(Eq. 2.1.)}$$

Release from suspension systems

$$Q = A * \sqrt{2 * D_s * C_0 * C_s * t} \qquad \text{(Eq.2.2.)}$$

Q= Cumulative amount of the liberated drug [mg]

A= Diffusion area [cm²]

C_0= Starting concentration of the drug in the donor [mg/cm³]

C_S= Saturation concentration of the drug in the donor [mg/cm³]

t= Time [s]

D_s= Diffusion coefficient (Liberation coefficient) of the drug [cm²/s]

A plot of Q/A versus square root of t should be linear; the diffusion coefficient is calculated from the slope.

In case of suspension bases following assumptions must be taken in consideration, to verify the equation 2.2.:

a) The suspended drug is in a fine state such that the particles are much smaller in diameter than the thickness of the applied layer.
b) The amount of drug present per unit volume in the donor is substantially greater than Cs.
c) Drug solubility in the receptor must be fast enough i.e. the dissolution rate should not be the rate-limiting step.
d) The receptor medium constitutes a perfect sink for the released drug i.e. the concentration of the drug in the receptor does not exceed 10% of its saturation concentration throughout the experiment.
e) After the lag-phase the diffusion rate changes only slightly.
f) The concentration gradient at the interface base/membrane is almost linear.
g) Only one drug diffuses through the base.

2.5. Factors affecting drug release

Drug release from semisolid preparations is known to be affected by a great number of factors, which can be summarized as follows:

- Cream base: The structure, the type as well as the composition of the cream base have a direct influence on drug release as all these factors influence the diffusion, distribution and solubility of the drug in the base (Poulsen et. al., 1968; Benninger, 1977).

- Thickness of cream base layer: The thickness of the base layer reflects the amount of drug applied. Therefore, increasing the thickness is accompanied by an increase in the diffused amount of drug (Horsch et al. 1974 b). However, if the thickness exceeds a certain limit the dose of the drug becomes infinite having thereby no more influence on drug release.

- Viscosity of cream base: The lower the viscosity of the preparation the easier for the drug to diffuse through the base to the interface (Korbar et al., 1982; Hsu et al., 1994; Fang et al., 1996)

- Volume and agitation of receptor medium: The drug liberation is increased by increased concentration gradient at the interface between donor and acceptor. This increase of the concentration gradient can be achieved through increasing the volume of the acceptor, shortening the sampling time (Horsch et al., 1974 b) or accelerating the speed of agitation of the acceptor. However, it should be taken in consideration that a high speed of agitation could disturb the uniform contact between the receptor medium and the membrane whereas a very slow circulation may result in increasing the thickness of the diffusion layer. An agitation of 400 rpm in the diffusion cell setup is recommended to achieve homogeneous and rapid mixing (Shah et al., 1999).

- Type of receptor medium: In most cases receptor medium is either water or physiological buffer system. However, for products containing water-insoluble drugs, selection of an appropriate receptor medium to maintain sink conditions

during in vitro release studies is a challenge. In order to achieve or improve drug release from such topical preparations, receptor media containing surfactants or different organic/aqueous solvents can be used (Shah et al., 1999).

- pH of acceptor medium: It affects the release of weak electrolytes. Changing the pH-value influences the partition coefficient of the drug and subsequently its liberation (Horsch et al., 1974 b).

- Temperature of experiment: Changing the temperature of the experiment has an influence on the consistency of the base as well as the solubility and diffusion coefficient of the drug. The experiments are normally carried out at 37°C which is the body temperature or at 32°C which represents the temperature of the skin (Horsch et al., 1974 a).

- Dispersion of the drug: The dissolution rate of drugs increases with increased dispersion. This relation is important for sparingly soluble drugs and has no significance with increased solubility of the drug in the base (Horsch et al, 1975).

- Solubility of the drug in the base: In suspension-type vehicles drug release is directly proportional to the amount of dissolved drug in the vehicle (Horsch et al., 1972).

- Drug concentration in the base: According to the equation of Higuchi (1967) the liberated amount of drug is directly proportional to the square root of the drug concentration in the base.

- Particle size: Particle size of the drug is inversely proportional to the dissolution rate and release, i.e. the smaller the size of particle the greater its rate of solubility in the base and subsequently its release (Loth et al., 1984).

2.6. Hazards of dilution of topical corticosteroid preparations

Extemporaneous dilution of topical corticosteroids formulations are used to be very commonly done in response of physicians' requests. The rational is that dilution enables the physician to restrict prescribing to well known corticosteroid molecules, while at the same time tailoring the formulation to the patients' need (Gao and Li Wan Po, 1994). Various authors have drawn attention to the dangers associated with this practice (Busse, 1978; Mooney, 1974; Smith, 1982).
The dangers of dilution stem from pharmaceutical, bacteriological and biopharmaceutical considerations.

Pharmaceutical considerations

These are mainly questions of physical compatibility of the product with the diluent and possible deleterious effects of the diluent on the active ingredient. The "External diluent directory " lists some of these incompatibilities (Busse, 1978):

- The FAPG (fatty alcohol propylene glycol) base will easily crack with many cream and ointment diluents, especially pastes or stiff ointments e.g. zinc ointment. Problems of drug stability can also occur.
- If betamethasone valerate cream is diluted with a cream base that has a neutral to alkaline pH, conversion of the 17-ester to the less active 21-ester may occur.
- The dilution of fluocinolone acetonide preparations with an unsuitable vehicle e.g. those containing oxidising agents, could cause chemical degradation of fluocinolone acetonide to less active compounds.

These are only a few of the pharmaceutical problems that can arise from dilution of topical steroid preparations. Another example reported in a previous investigation showed a larvated incompatibility upon diluting Topisolon® ointment with Unguentum Cordes, both having different types of emulsifiers. Macroscopically, the formulation appeared homogeneous, however microscopical examination revealed large water drops indicating coalescence of the emulsion (Wolf, 1988).

Bacteriological considerations

During dilution of topical steroid preparations, pathogenic bacteria may be introduced and could infect the diseased skin to which the preparation is applied. That could be especially hazardous to the patient because there is ample clinical evidence that steroids help the spread of infection. An organism of major importance in that respect, particularly in hospital environment, is Pseudomonas aeruginosa, which is resistant to most antibiotics. It can survive and multiply outside the human body and cause skin lesions. In 1966 an epidemic strain of Pseudomonas was isolated from skin lesions of patients to whom a diluted steroid had been applied. Pseudomonas aeruginosa was found in the diluted cream (Busse, 1978).

Furthermore, on dilution the preservatives may be inactivated due to an incompatibility or simply by being diluted out to below their effective levels. Many of the preservatives commonly used in pharmaceutical emulsions are absorbed or bound by non-ionic emulsifiers and this bound preservative is devoid of antimicrobial activity.

An additional factor is the partition coefficient of the preservative. The degree of preservation depends on the availability of the preservative in the aqueous phase. If, on dilution of a well preserved cream, further oils are added, the preservative may tend to partition into that oil phase leaving the product in a badly preserved state (Mooney, 1974).

Thus, for complete microbiological safety, topical preparations would need to be diluted under aseptic conditions with sterile diluent, which would be both inconvenient and very costly.

Biopharmaceutical considerations

Marketed formulations of topical corticosteroids result from extensive research of the optimum concentration and most suitable base for therapeutic effect. The release of steroid from a topical preparation is dependent on the nature of the vehicle and therefore dilution with an inappropriate base can lead to the wrong conditions for adequate release. Moreover, diluting a corticosteroid preparation containing an antimicrobial agent will most likely reduce it to below its effective concentration. Apart from the therapeutic disadvantage, in the case of antibiotics this could encourage the development of resistant organisms (Busse, 1978).

The only significant argument in favour of diluting potent proprietary preparations is that if they are to be used in large quantities by the patient and if the diluted preparation is shown to be equivalent to the undiluted product in terms of clinical efficacy then the danger of adverse reactions may be lessened by using the diluted preparation. Even in this latter situation, however, an adequate knowledge of the potency likely to result from dilution would normally lead one to be able to select an undiluted proprietary product of appropriate strength.
Furthermore, as the clinical efficacy of topical corticosteroids is, in most cases, paralleled by their ability to produce local side effects a degree of dilution which results in similar clinical efficacy may well not reduce this problem. Moreover, it should not be too readily assumed that dilution always leads to a smaller risk of significant systemic absorption and systemic side-effects (Gibson et al, 1983).

Dilution will, however, continue despite these comments; therefore, regulations and precautions must be put down to control these dilutions in order to protect the interests of both the prescriber and the patient. Some suggestions were made by Mooney (1974):

1. Use simple, adequately preserved diluents
2. Make small batches
3. Do not keep longer than necessary for patient treatment
4. Use aseptic techniques for mixing

2.7. The structure of the skin

Skin is essentially composed of two major layers: an outer, unvascularized epithelial layer (the epidermis), and an inner vascularized layer (the dermis).

Stratum corneum

The stratum corneum constitutes the superfacial layer of the epidermis. It is the final stage of differentiation and is formed from several layers (10-25) of dead cells embedded in a lipid matrix. The stratum corneum is about 10 µm thick in the non-hydrated state. In hand palms and foot soles the stratum corneum is much thicker, about 400-600 µm (Barry, 1983).

On a dry weight basis, the stratum corneum is mostly protein (75-80%) with the greater part being fibrous intracorneocyte α-keratin having a small fraction of amorphous β-keratin (Matoltsy et al., 1968). Together, these components make the corneocyte dense and almost impermeable for solutes. Approximately 10-15% of the protein fraction is water soluble. The remainder of the stratum corneum consists of a complex lipoidal mixture (5-15%) and unidentified material (5-10%) (Suhonen et al., 1999).

The viable epidermis

Situated beneath the stratum corneum is the remainder of the epidermis, the viable epidermis. It ranges in thickness from 75-150 µm and consists of various layers, characterized by various stages of differentiation. When going inwards, the penetrating chemical successively crosses the stratum lucidum, the stratum granulosum (or granular layer), the stratum spinosum (or spinous layer) and the stratum basale (or basal layer).
In the basal layer, the cells continuously undergo mitoses to renew the epidermis and in healthy skin, this proliferation compensates the desquamation of dead horny cells from the skin surface.
The cellular structure of the viable epidermis is predominantly hydrophilic throughout its various layers and substances can be transported in its intercellular fluids. Especially for polar substances, the resistance to permeation is considerably lower than in the stratum corneum (Schaefer et al., 1982).

Dermis

Chemicals finally reach the dermis, a 3-5 mm thick layer, which contains a rich supply of capillaries, nerves, sweat glands, sebaceous glands, and hair follicles that are supported by connective tissue (Barry, 1983).
Apart from some radial transport in the dermis by the capillaries of the cutaneous microvasculature, chemicals are readily absorbed into the blood stream and diluted systemically. The blood stream enables the dermis to act as a sink for molecules that finally reach this layer. This ensures a maximal concentration gradient of the penetrant across the epidermis.

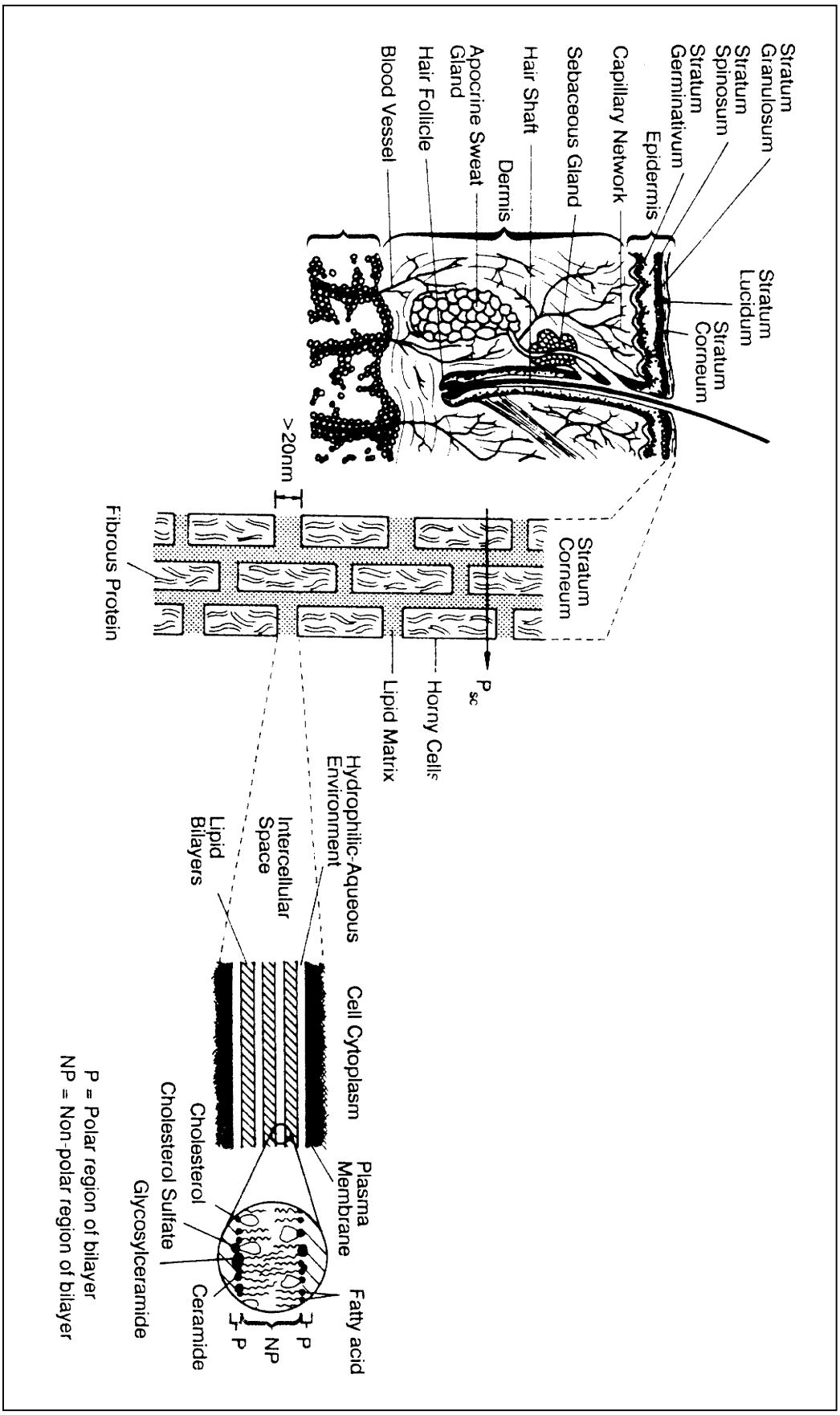


Fig. 2.2. A cross sectional view of human skin (Gurny and Teubner, 1993)

2.8. Topical application of drugs

Topical application of drugs for systemic therapy (Wiechers, 1989) may have several advantages over the conventional oral route.

- It circumvents variables that may influence the gastro-intestinal absorption, such as the drastic changes in pH along the gastrointestinal tract, food intake, intestinal motility, etc. Additionally it may eliminate systemic first-pass metabolism as it circumvents the liver. Moreover, delivering drugs by the transdermal route may provide controlled, constant administration of the drug allowing continuous input of drugs with short biological half-lives.

Transdermal therapy, however, also has its limitations. Firstly, and foremost, the skin acts as a barrier in two directions, controlling the loss of water, electrolytes, and other body constituents, while preventing the entry of drug molecules as well as harmful or unwanted molecules from the external environment. Secondly, there may be pharmacodynamic, physiological and/or physico-chemical limitations. Compounds may act as irritants (sodium lauryl sulfate); may cause allergic sensitization (some antibiotics), hyperpigmentation (bliomycin), or be keratolytic (salicylates).

Percutaneous absorption is the uptake of a compound into the systemic circulation after dermal application. It can be divided into three steps:

- Penetration, which is the entry of a substance into a particular layer or organ
- Permeation, which is the penetration through one layer into another which is both functionally and structurally different from the first layer.
- Absorption, which is the uptake of a substance into the vascular system (lymph and/or blood vessel), which acts as the central compartment.

Factors that influence the percutaneous absorption of chemicals through the skin are:

- The structure of the skin
- The physico-chemical characteristics of the penetrant
- The physico-chemical characteristics of the vehicle in which the penetrant is dosed
- The dosing conditions

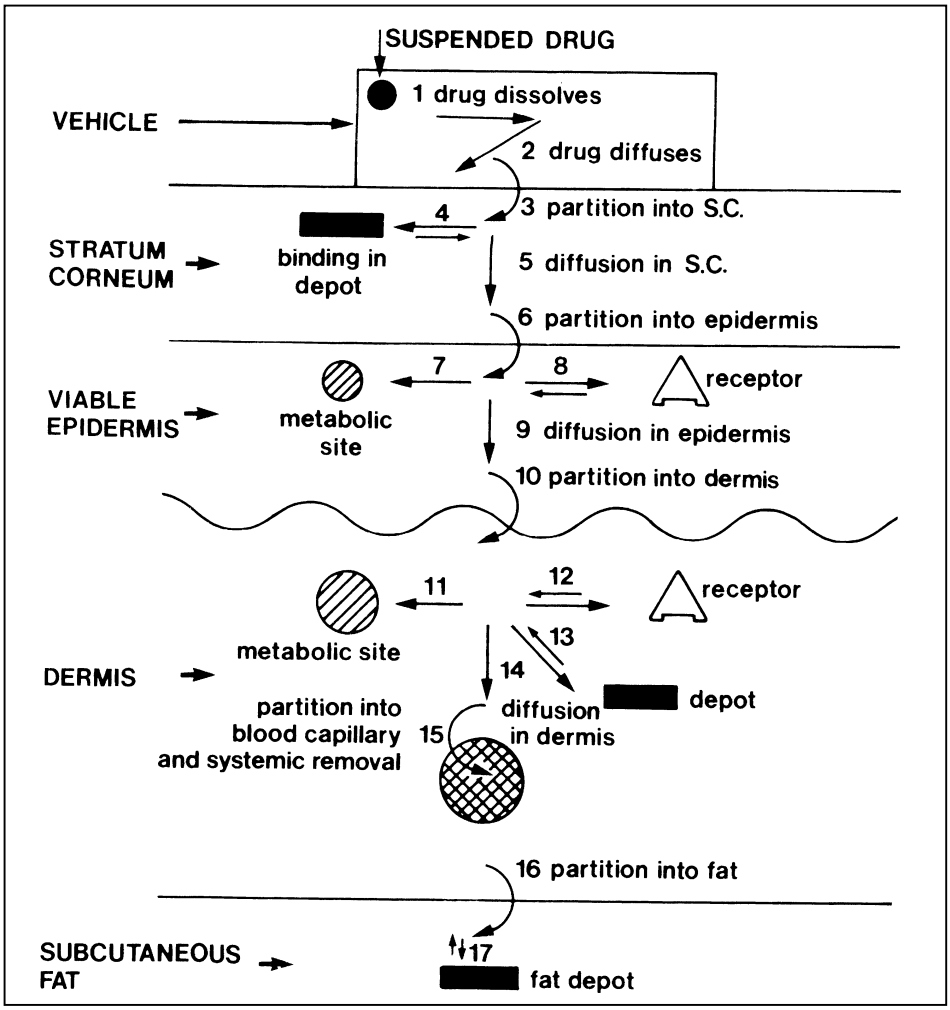


Fig. 2.3. Some stages in percutaneous absorption from a suspension base (Barry, 1983)

The Fig. 2.3. represents a simple idealization of the drug flux which may arise clinically following the application of topical suspension vehicles. The drug particles must first dissolve so that molecules may diffuse within the vehicle to reach the vehicle-stratum corneum interface. For the drug to move through the skin it must partition into the stratum corneum and diffuse within this very impermeable barrier. Some drugs may bind at a so called depot site; the remainder diffuses in the horny layer, meets a second interfacial barrier and partitions into the viable epidermis. Whereas the initial partition process may have favoured an increased flux (for lipophilic drugs), the second partitioning will be unfavourable as the viable epidermis provides a more hydrophilic milieu compared with the stratum corneum. Any substance with a high affinity for the horny layer and a very low water solubility may not be absorbed percutaneously even though it may have penetrated the barrier

layer. The drug then partitions into the dermis. Within the dermis, additional depot regions and metabolic sites may intervene in the progress of the drug to a blood capillary. A fraction of the diffusant may also partition into the subcutaneous fat to form a further depot (Barry, 1983).

2.9. The stratum corneum barrier function

The barrier function of the skin is firmly attributed to the stratum corneum. The stratum corneum has a water permeability approximately 1000 times lower than most other biological membranes, which has been attributed to the unique lipid composition and content of the stratum corneum and, especially, the exceptional structural arrangement of the intercellular lipid matrix and the envelope surrounding the corneocytes (Potts and Francoeur, 1991).

The essential role of the stratum corneum lipids in barrier properties has been demonstrated by removal of lipids with solvent extraction, which leads to increased transepidermal water loss and enhanced skin permeability (Sweeney and Downing, 1970). In addition, it has been found that impaired barrier function in diseased skin is related to abnormalities in stratum corneum lipid composition (Imokawa et al., 1991) and that the recovery of the barrier function can be achieved by topical application of stratum corneum lipids. The stratum corneum water permeability has been shown to be directly correlated with lipid alkyl chain conformation (Golden et al., 1987 b).

2.10. Stratum corneum lipid composition

The lipid composition of the stratum corneum is highly unique. It is virtually devoid of phospholipids, unlike other biological membranes, and is mostly composed of ceramides (41%), cholesterol (27%), cholesteryl esters (10%) and fatty acids (9%) with a small fraction of cholesterol sulfate (2%) (Wertz and Downing, 1989). The lipid composition and content vary with body site. A structurally heterogeneous group of seven different ceramides represents the major polar lipids present in the stratum corneum. This high content of ceramides, having long, straight, saturated aliphatic chains would seem ideally suited for the formation of highly ordered, impermeable

membranes, which provide resistance to temperature variations, UV-exposure, and air oxidation (Schurer and Elias, 1991).

2.11. Stratum corneum lipid arrangement and phase behaviour

The extracellular lipids between the corneocytes are arranged in multiple lamellar structures forming continuous lipid phases that occupy approximately 20% of the total stratum corneum volume (Elias and Leventhal, 1979). The lamellar arrangement is mainly due to the ceramides, which may provide the polar moieties required for such arrangements and are capable for extensive hydrogen bonding. In addition, it has been suggested that sufficient amphiphatic material exists in the stratum corneum lipid fraction such as cholesterol sulfate that maintains the lamellar form (Williams and Elias, 1987). The lamellae form oriented structures aligned parallel to the surface of the stratum corneum (Garson et al., 1991).
Given the considerable chain-length diversity among stratum corneum lipids and, on the other hand, the mechanical properties required of the bilayers, a domain mosaic model was recently proposed involving segregation of lipids as domains of crystalline/gel phase lipids bordered by lipids in a liquid crystalline phase provided by relatively short chain lipids (Forslind, 1994).

2.12. Drug permeation routes

The absorption of the drug through the skin is believed to be passive (Scheuplein and Blank, 1971). Transdermal permeation can involve passage of molecules across the intact epidermis or through a shunt pathway offered by the relatively universally distributed hair follicles and eccrine glands. The latter route is considered to be of minor importance because these skin appendages actually occupy only 0.1% of the total human skin surface. In addition, they exercise some influence on percutaneous absorption due to their secretions which affect the lipid and the water content of the stratum corneum and so can modify the absorption of molecules (Hueber et al., 1992). Therefore, it is now widely believed that the transepidermal pathway of passive diffusion is the principal pathway associated with the permeation of drugs

through the skin. But for molecules, due to their large molecular size, for example, the shunt pathway can be significant (Hueber et al, 1994).

Percutaneous absorption via the transepidermal pathway involves diffusion through the stratum corneum and the viable cell layers of epidermis, and finally through the upper layers of dermis into the microcirculation (Squier et al., 1991). The rate determining step in the percutaneous absorption of most drugs is their permeation across the stratum corneum providing the major portion of resistance (Scheuplein and Blank, 1971). Only for very lipophilic drugs the essential aqueous nature of the viable epidermis may provide a significant barrier.

The stratum corneum is often characterized as a brick wall-like structure with the corneocytes (protein) forming the bricks and the intercellular lipid as a mortar (Elias, 1983). The protein phase is discontinuous while the lipid phase is continuous. Through the stratum corneum, in theory, there exist two potential pathways; the transcellular (across the corneocytes and the lipid matrix) and the intercellular (via the lipid domains between the corneocytes).

However for both routes the structure of the stratum corneum dictates that the permeants must diffuse across the intercellular lipid layers. The intercellular route is widely believed to provide the principal pathway for the permeation of most drugs. Experimental studies have shown that the permeation of most compounds is strongly dependent on their lipophilicity and molecular size (Guy and Hadgraft, 1988). However, it has been suggested that only very hydrophilic compounds penetrate through both the hydrophilic regions of the corneocytes (presumably water associated with keratin) and the lipid matrix (Flyn, 1989).

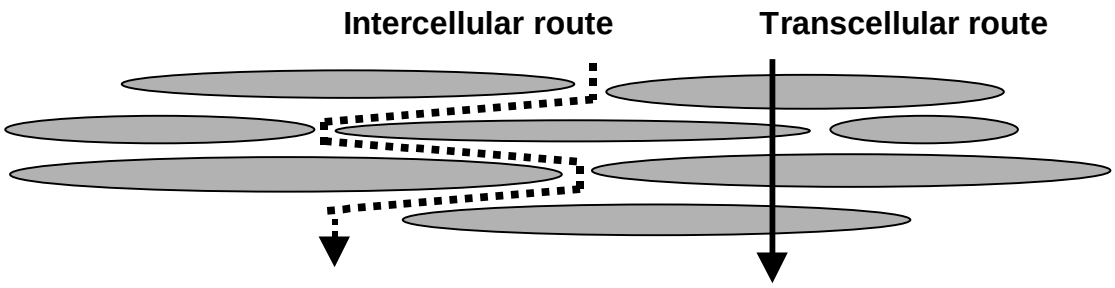


Fig. 2.4. A scheme representing suggested routes for drug permeation in the stratum corneum (i.e., (i) across the corneocytes and the intercellular lipid matrix (the transcellular pathway) and (ii) via the lipid matrix between the corneocytes (the intercellular pathway). (Barry, 1987 a)

The amount of drug permeated through the skin per unit time and unit area is defined as the drug-flux J. In the case of perfect sink the flux is calculated after the equation 2.5. (Flynn et al., 1974) which is based on Fick's 1. law for drug diffusion (equation 2.4.).

$$\frac{dm}{dt} = -q * D * \frac{dc}{dx} \qquad \text{Eq. 2.4.}$$

$$J = \frac{dm}{dt * A} = \frac{D_s * C_0 * K}{h} \qquad \text{Eq. 2.5.}$$

$\frac{dm}{dt}$ = Mass flow

J = Drug-flux [g/cm²*s]

A = Permeation area [cm²]

D_s = Apparent diffusion coefficient in the skin [cm²/s]

C_0 = Starting concentration of the drug in the donor [g/cm³]

K = Partition coefficient skin/ donor

h = effective skin thickness [cm]

The partition coefficient as well as the effective skin thickness are difficult to be determined; therefore, they are summarized together with the apparent diffusion coefficient to the permeability coefficient [cm/s] in equation 2.6..

$$P = \frac{D_s * K}{h} \qquad \text{Eq. 2.6.}$$

Equation 2.5. is thus simplified to 2.7. :

$$J = P * C_0 \qquad \text{Eq. 2.7.}$$

-

In suspension vehicles only the dissolved part of the drug is able to diffuse through the skin; therefore it seemed important to calculate another coefficient that makes a

relation between the flux and saturation concentration of the drug in the vehicle. This coefficient is called per-sol-coefficient Z [cm/s] and is calculated as illustrated in equation 2.8.

$$Z = \frac{J}{C_s} \qquad \text{Eq. 2.8.}$$

2.13. Testing of percutaneous absorption

In vitro methods

- Excised stratum corneum: Stratum corneum is the principal barrier of the skin for penetration of most drugs; therefore, it is suitable for testing the percutaneous absorption. Besides, the stratum corneum being a dead tissue, enables its storage after isolation for several months without being affected, thus makes it possible to test various compounds over a long period using the stratum corneum of the same donor.

- Excised skin: Using the whole skin is beneficial in testing very lipophilic drugs for which the aqueous epidermis and dermal layers may provide the significant hindrance for diffusion (Barry, 1987 a). Furthermore, the excised skin may be also useful to test the percutaneous absorption of compounds, which are mainly metabolised in the skin. However, excised skin must be used directly after preparation in order to maintain the viability and enzymatic activity of the tissue, which may be regarded as a limitation of this method.

- Stratum corneum lipid model: The impermeability of the stratum corneum to most drugs is mainly attributed to its unique lipid composition; therefore reconstructing this lipid composition in the same proportions as in the stratum corneum could be successfully used to test the percutaneous absorption (Glombitza, 2000). This method has the advantage that it overcomes the difficulties in obtaining and preparing the skin or stratum corneum; moreover, it overcomes the interindividual variations of the donors (age, sex, skin region etc.).

- Cell cultured skin: Another alternative to standardize the percutaneous absorption testing and to overcome the interindividual variations is to construct a model of the human skin by using cell culture. Using human keratinocytes and fibroblasts it was possible to culture an artificial human skin that has comparable permeation properties to excised human stratum corneum (Specht, 1999); furthermore, it is again useful in testing the permeability of drugs that are principally metabolized in the viable epidermis.

In vivo methods

- Systemic bioavailability: Percutaneous absorption in vivo is usually determined by an indirect method based on the measurement of radioactivity in excreta following topical application of a labeled compound. The usage of radioactive compounds (usually carbon-14- or tritium labeled) is necessary in human studies because the plasma levels of the compound are extremely low following topical application often below assay detection level (Wester et al., 1983). However this method is only suitable to detect the systemic effect of a topically applied compound and not a local one.

- Stripping method: The stripping method determines the concentration of a chemical in the stratum corneum at the end of a short application period (30 minutes). The stratum corneum is removed by successive tape application, the tape strippings are then assayed for chemical content. Rougier et al. (1987) have established a linear relationship between this stratum corneum reservoir content and percutaneous absorption using the standard urinary excretion method.

- Biological response: Another approach used to determine in vivo percutaneous absorption is to use a biological/pharmacological response. This method is principally applied for compounds which elicit an easily measurable response. An example of biological response would be the vasoconstrictor assay of corticosteroids. This assay uses the skin blanching effect of the corticosteroids to estimate the rate and extent of drug diffusion to the dermal vasculature, the intensity of this whiteness correlates directly with the topical availability of the drug (Mc Kenzie and Stoughton, 1962).

2.14. Mode of action of penetration enhancers in human skin

A major problem in attempting to control the drug flux arises from the impermeability of human skin and its biological activity. It would be very useful to circumvent these problems by including in the formulation molecules which would reversibly remove the barrier resistance of the stratum corneum and thus allow the drug to penetrate to the viable tissues and enter the systemic circulation. Such entities are known as penetration enhancers.

Interactions between penetration enhancer and the lipid bilayer structure can occur at three places as illustrated in Figure 2.5., i.e. at the polar head groups of the lipid bilayer (site A), within the aqueous regions between the polar lipid head groups (site B) and between the hydrophobic tails of the bilayer (site C) (Barry, 1987 b).

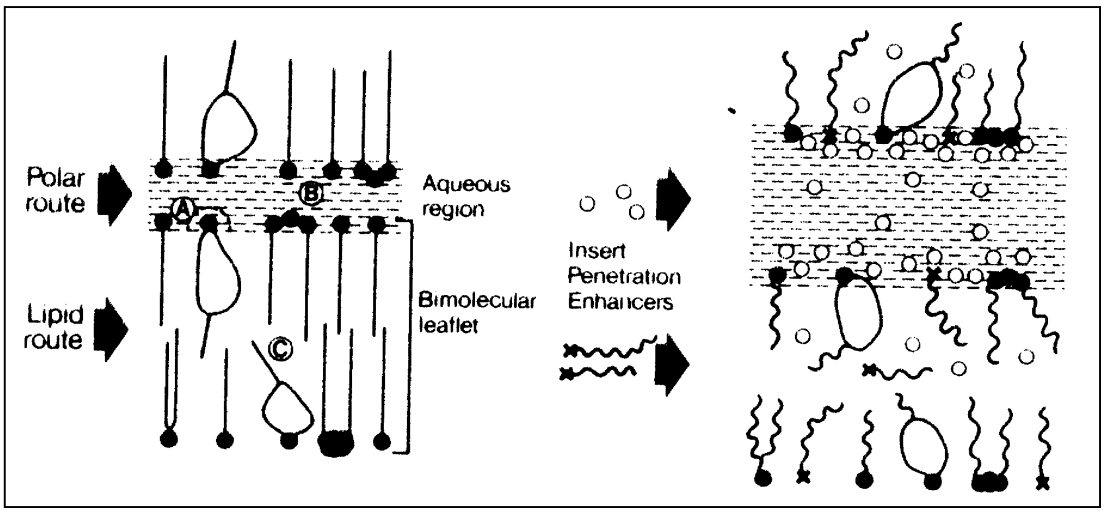


Fig. 2.5. Postulated sites of action for penetration enhancers in the intercellular domain, illustrating the change from relative order to relative disorder after the insertion of penetration enhancers. Small circles represent polar enhancers such as propylene glycol and ethanol; linear chain molecules represent Azone and oleic acid molecules (Barry, 1987 b).

Water and dimethyl sulfoxide are examples of compounds that interact with the polar head groups of the lipid bilayer (site A). The presence of water molecules results in hydration shells around the polar head groups via hydrogen bonding. The hydration

spheres of the lipids will then be disturbed which loosens its packing. This results in a more fluid and more permeable hydrophobic route (Bennet and Barry, 1985).
Penetration enhancers such as propylene glycol and ethanol exert a direct influence on the aqueous regions between the polar lipid head groups of the bilayer (site B). These enhancers penetrate into this region of the tissue in such amounts that they alter the solubilizing ability of this site; thereby promoting drug partitioning into the skin which subsequently results in an increased flux of penetrant (Bennet and Barry, 1985).

On the other hand, penetration enhancers like Azone® and unsaturated long chain fatty acids such as oleic acid exert their action on the hydrophobic tails of the bilayer (site C) by upsetting their packing and increasing their fluidity, thus permitting easier diffusion of penetrants. Concomitantly, these alterations in the lipid packing may also affect the rigidity of the polar head group region (Goodman and Barry, 1986).

The key to alter the transcellular route, i.e. the polar pathway, will be to swell the protein matrix or to change its structure. Such processes are to take place within the corneocytes at the keratin fibrils and their associated water molecules by interaction with polar groups, relaxation of binding forces and alteration in the conformation of the helices, but the enhancer may also interact with whatever lipid remains in the corneocyte. Extensive interaction may even result in pore routes. Examples of penetration enhancers acting on polar pathway include pyrolidones and surfactants (Bennet and Barry, 1985).

2.15. Microstructure of stratum corneum

The barrier properties of the stratum corneum are attributed mainly to its very complex lipid composition which is characterized by having a high degree of order and high density which explains the low diffusion coefficient in this medium (Loth, 1989). In order to relate the barrier function to stratum corneum structure the investigation of the structure of stratum corneum by means of several techniques like differential scanning calorimetry (DSC), small and wide angle X-ray diffraction (SAXD & WAXD), electron spin resonance (ESR) or Fourier transform infrared spectroscopy

(FTIR) seemed to be very important. Thus any alterations in the permeability of the stratum corneum could be then referred to possible structural changes.

2.15.1. Differential scanning calorimetry (DSC)

Four transitions are observed in the DSC thermal profile of human stratum corneum between 25-105°C. The transitions occur near 35, 65, 80 and 105°C (Van Duzee, 1975; Golden et al., 1987 b; Hiroven et al., 1994).
The highest temperature transition near 105°C (T4) is due to the thermal denaturation of intracellular keratin. This peak is not thermally reversible as expected for a protein denaturation. Furthermore, it is present following solvent treatment of the stratum corneum, a process known to remove intercellular lipids with little or no extraction of highly cross-linked keratin. Finally, this transition is absent from a corneocyte membrane preparation, which is lacking intracellular keratin due to exhaustive alkali extraction.

The third temperature peak near 80°C (T3) exhibits thermal properties characteristic to both proteins and lipids. First, this transition is not thermally reversible which is an indication for the presence of protein. Nevertheless, the Tm decreases to a limiting value with increasing water content, a behaviour characteristic of numerous water-lipid systems. In addition, treatment of the sample with lipid solvents removes the transition from the thermal profile, proving lipid contribution to the peak. It is quite possible that this peak results from a thermal transition of a corneocyte membrane protein-intercellular lipid complex. According to this model, the intercellular lipids near the membrane boundary are stabilized by nearby proteins.

The second temperature peak near 65°C (T2) is due to thermal transitions in the intercellular lipids. This consideration is ensured by the thermal reversibility of the transition. Furthermore, increasing water content results in a decrease in the transition temperature to a limiting value. In addition, the peak is present in a corneocyte membrane preparation and hence not associated with intracellular keratin. Finally, the peak is absent in chloroform-methanol-treated samples, a technique known to extract intercellular lipids (Golden et al., 1987 b).

The 35°C thermal transition (T1) is small and its occurrence was found to vary from sample to sample. This peak is always thermally reversible and thus appears lipid in nature. The small and variable nature of this peak suggests that the lipid is not tightly associated with the stratum corneum and may arise from sebaceous lipids still adhering to the surface after hexane washing (Golden et al., 1986).

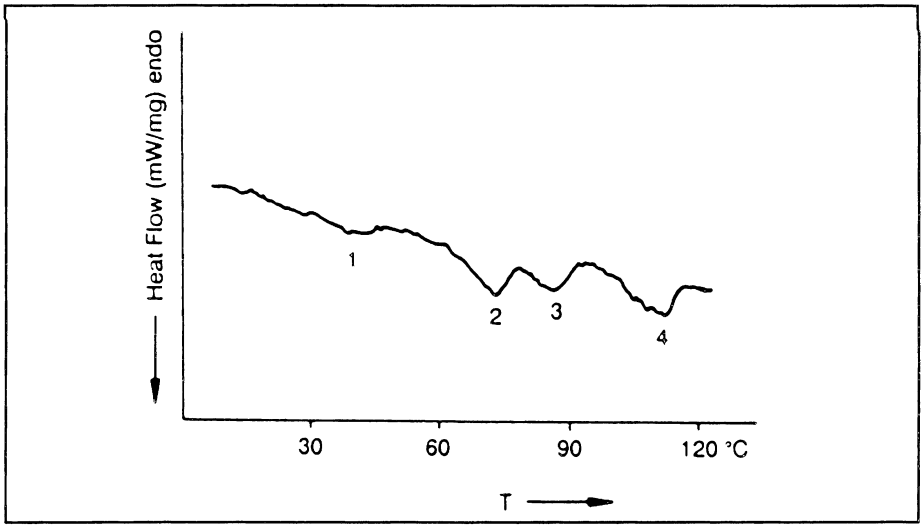


Fig. 2.6. Thermogram of human stratum corneum (Bouwstra et al., 1993)

2.15.2. Wide-angle X-ray diffraction (WAXD)

WAXD investigations of human stratum corneum reveal two very strong reflection rings at 0.378 and 0.417 nm, which are probably due to crystalline lipids (Small, 1986). These two reflections are characteristically produced by crystalline lipids with an orthorhombic perpendicular alkyl chain backing arrangement (Bouwstra et al., 1992). However, the reflection at 0.417 nm is also characteristic of gel phase lipids with a slightly looser hexagonal alkyl-chain packing arrangement. It is possible, therefore, that both crystalline and gel phase lipids may coexist in the stratum corneum, i.e. there may be lateral phase separation in the bilayers (Cornwell et al., 1994).

It was noticed that after lipid extraction the reflection at 0.417 exists though of very low intensity, which was referred to the linkage of these lipids to the corneocyte envelope (Bouwstra et al., 1992).

Two broad diffraction rings at 0.46 and 0.98 nm are also observed. The reflection ring at 0.46 nm is probably due both to an orientational order of hydrocarbon chains in the liquid state and to soft keratin located in the corneocytes. The reflection ring at 0.98 nm is referred only to soft keratin. The broad non-uniform band indicates a slight orientation (Bouwstra et al., 1992).

3. Materials and methods

3.1. Materials

3.1.1. Cream base ingredients

The following materials were used to prepare the bases investigated in this study. All substances were used from only one batch to exclude the influence of any batch variations.

- Emulsifying cetostearyl alcohol (Henkel, D-Düsseldorf)
  It consists of 10% (m/m) sodium cetostearyl alcohol sulfate and 90% cetostearyl alcohol. The content of sodium cetostearyl alcohol sulfate in the utilized charge was 9.9%. The hydroxyl number was 203.

- Cetostearyl alcohol (Caesar & Loretz GmbH, D-Hilden)
  The used batch obeyed the instructions of DAB 9 for properties and purity.

- White petrolatum (Hansen & Rosenthal, D-Hamburg)
  The congealing point was 54.5°C

- Liquid paraffin DAB 10 (Mainland, D-Frankfurt)
  The density was 0.865 (g/cm^3)

- Wool fat alcohol (Caesar & Loretz GmbH, D-Hilden)

- Glycerol 85% (Henry Lamotte GmbH, D-Bremen)

- Tween® 60 (ICI, UK-Cleveland)

- Isopropyl myristate (Merck, D-München)
  The density was 0.853 g/cm^3.

3.1.2. Water

Water was used in bidistilled quality.

3.1.3. Active Ingredient

Micronized hydrocortisone (Synopharm, D-Hamburg) was used in Pharmacopoea quality (DAB 10). The content determined per HPLC by Synopharm was > 99%.
Structure and further informations are given in 2.1.

3.1.4. HPLC-Mobile phase

Methanol in HPLC quality from the companies Fluke (D-Neu-Ulm) and J.T. Baker (NL-Deventer) was used.

3.1.5. Buffer

The phosphate buffer pH 7.4 was prepared in accordance to phosphate buffer solution pH 7.4 containing sodium chloride DAB 1997. However, sodium chloride was found to disturb the HPLC analysis therefore the buffer was prepared without adding sodium chloride i.e. 2.38 g sodium monohydrogen phosphate (Merck, D-Darmstadt) and 0.19 g potassium dihydrogen phosphate (Merck, D-Darmstadt) were dissolved in one litre bidistilled water. The pH-value of the buffer was adjusted to 7.4 by adding potassium dihydrogen phosphate.

3.1.6. Commercial products

Soventol® Hydrocortison Creme and its placebo (Knoll Deutschland GmbH, D-Mannheim)

3.2 Methods

3.2.1. High performance liquid chromatography (HPLC)

The HPLC system consisted of a gradient pump Beckman System Gold Solvent Delivery system 126 (Beckman, D-München) and a UV-Detector Beckman System Gold Detector Module 166 (Beckman, D-München).
The peak identification and analysis were performed by the Beckman Gold Chromatography Software Version 6.01 (Beckman, D-München).
A reversed phase column (250 x 4 mm) was used, it was filled with Hypersil ODS 5 µm (Grom, D-Herrenberg).
The mobile phase was methanol/water in the ratio 60:40 with a flow rate of 1.1 ml/min (Way and Hadgraft, 1991). The resulting pressure was 280-290 bar.
The retention time for hydrocortisone was 4.8 minutes at a wave length of 250 nm. Linear correlation between peak area and hydrocortisone concentrations was obtained within the concentration range of 0.1 µg/ml-20 µg/ml. The correlation coefficient was > 0.999.

3.2.2. Differential scanning calorimetry (DSC)

Human stratum corneum as well as the cream bases were thermally analyzed using a Differential Scanning Calorimeter DSC 220 C with a disc station 5200 H (Seiko, J-Tokyo). Stratum corneum pieces were folded in an aluminium crucible (SSC000C008, C3 Analysis Technique, D-Baldheim) and fused on cold. The probes were measured against empty reference crucible in a temperature range of –20°C to 140°C with a heating rate of 5°C/min. The weight of the hydrated stratum corneum pieces and the cream base probes was about 12 mg and 6 mg respectively.

3.2.3. Wide angle X-ray diffraction (WAXD)

Wide angle X-ray measurements of hydrated human stratum corneum were done using Debeye-Scherrer Camera (circumference 360 mm).
Small pieces of stratum corneum were carefully brought in an x-ray amorphous glass capillary (d = 0.5 mm) (Glas, D-Berlin) and were measured for about 96 hours.
Settings of the equipment:

X-ray generator	PW 1830 (Philips, D-Kassel)
X-ray tube	PW 2253/11 (Philips, D-Kassel)
Accelerating voltage	40kV; anode current 40mA
Radiation	CuKα, (λ = 0.154 nm)

In order to visualize the diffraction rings, the special film material Fuji 100 (Fuji, J-Tokjo) was blackened.
The diffraction rings were calculated using Bragg's equation:

$n\lambda = 2 * d * \sin\theta$

λ = wave length; d = interlayer spacing; θ = angle of diffraction

3.2.4. Determination of saturation concentration of hydrocortisone

3.2.4.1. Determination of saturation concentration of hydrocortisone in buffer

It is very important to determine the saturation concentration of the drug in the acceptor phase of the release as well as the permeation experiments in order to be able to ensure sink conditions. Sink condition exists when the diffused amount of the drug in the acceptor phase (phosphate buffer) does not exceed 10% of its saturation concentration.
The saturation concentration of hydrocortisone in phosphate buffer was determined by adding excess hydrocortisone to phosphate buffer. The suspension was stirred using a magnetic stirrer (300 rpm) over 24 hours at 20°C. Afterwards, the suspension was filtered through cellulose acetate filter (pore size 0.22 µm, Sartorius, D-Göttingen). The concentration of the hydrocortisone in the filtrate was then measured using the UV-210 A Spectrometer (Shimadzu, J-Kyoto) at a wave length of 250 nm.

Linear correlation between absorption and hydrocortisone concentration was obtained within the concentration range 2.5 µg/ml-10 µg/ml.

3.2.4.2. Determination of saturation concentration of hydrocortisone in cream bases

The base was prepared with a definite concentration of hydrocortisone; after having been left for 3 days at room temperature the preparation was examined for the presence of hydrocortisone crystals using a polarizing microscope, Zeiss Photomicroscope III (Zeiss, D-Oberkochen). If no crystals were detected, higher concentrations were examined, until crystals could be found. The first concentration at which crystals could be detected was taken as concentration at saturation (Loth, et al., 1979).

3.2.5. Determination of density of cream bases

The determination of the density was necessary to convert the concentrations from w/w into w/v in order to fulfil the requirements of the Higuchi equation. The density of the different cream bases was determined at room temperature using a Beckman Air Comparison Pycnometer 930 (Beckman, D-München). The weight of the probes was about 10 g.

3.2.6. Determination of the emulsion type of cream bases

3.2.6.1. Colouring method

The emulsion type was determined by sprinkling a lipophilic (Sudan red Merck, D-Darmstadt) and a hydrophilic colouring agent (Methylene blue Merck, D-Darmstadt) on the base. The colouring agent colours the corresponding coherent phase.

3.2.6.2. Conductivity method

The conductivity was measured by a temperature controlled standard conductivity measuring cell Tetra Con 96 (Wissenschaftliche Technische Werkstätten WTW, D-Weilheim) with a microprocessor conductivity meter (WTW, D-Weilheim).

3.2.7. Determination of pH-value

The pH-value of the buffer solution was measured by a pH-meter pH 539 (Wissenschaftliche Technische Werkstätten WTW, D-Weilheim). The calibration was done by standard buffer solutions pH 4.66 and pH 9.00 at room temperature.

3.2.8. Release experiments

The release experiments were performed in modified Franz diffusion cell (Franz, 1975) (Fig. 3.1.)

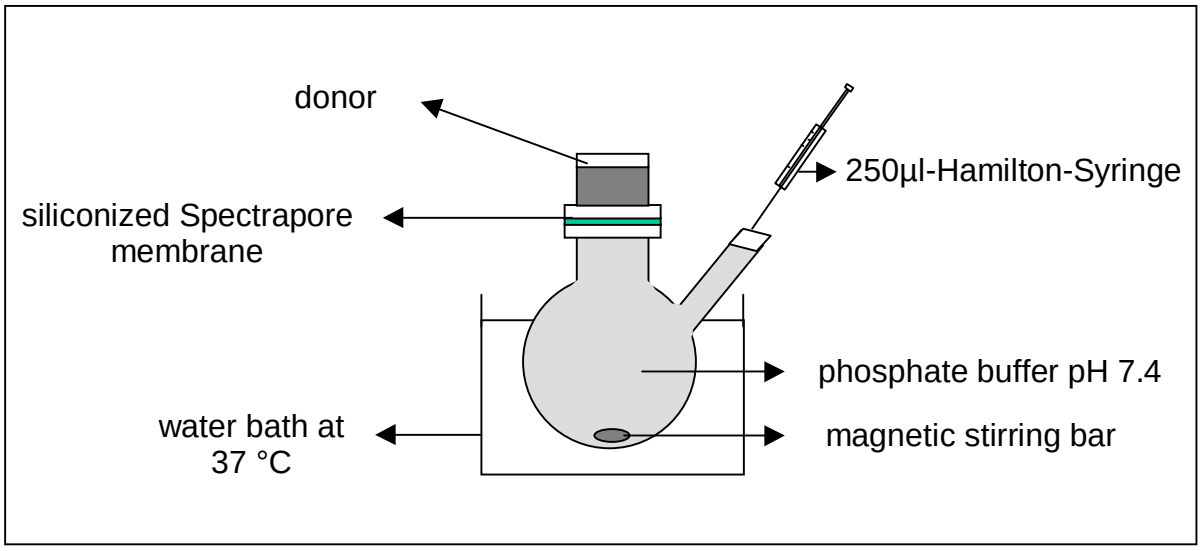


Fig. 3.1. Modified Franz diffusion cell

The experiments were carried out in triplicate. The donor compartment was filled with the formulation and phosphate buffer pH 7.4 (3.1.5.) was used as acceptor. The utilized cells had a liberation area ranging from 0.47 to 0.59 cm^2 and an acceptor volume varying between 4.4 and 6.5 ml. A proper homogenization of the released drug in the acceptor throughout the experiment was achieved by a rotating magnet (400 rpm).

Donor and acceptor were separated from each other by a Spectrapore membrane MWCO 6000-8000 (Spectrum Medical Industries; USA Los Angeles). In order to prevent the diffusion of water through the donor phase, the membrane was impregnated by polymethylsiloxane. This was done by initially hydrating the membrane in water then soaking it for 2 minutes in a 2% m/m solution of silicone oil AK 350 (Wacker, D-München) in diethyl ether (Merck, D-Darmstadt). The membrane was then stretched on the Franz cell and brought in horizontal position to ensure homogeneous thickness of the silicone film. Finally, 0.5 ml of the silicone oil solution was applied to the top of the membrane and kept over night under the hood to evaporate the ether thereby ensuring a final concentration of 1.2 mg silicone polymer per cm^2 of liberation area.

The acceptor compartments of the Franz cells were mounted in a water bath at 37°C, whereby the membranes have a temperature of 32°C which is comparable to the physiological temperature of the skin surface. The duration of the experiment was 7 hours. Aliquots of 250 µl using a 250 µl-Hamilton-Syringe 1725 RN (Hamilton, CH-Bonaduz) were taken every 30 minutes in the first 2 hours, then every 60 minutes and replaced by fresh buffer. The amount of hydrocortisone in the acceptor medium was analyzed by HPLC (3.2.1.).

3.2.9. Excised human stratum corneum

3.2.9.1. Isolation of stratum corneum

Healthy skin gained in plastic surgeries from the abdominal region of female donors was ultilized. Immediately following excision the skin was cooled and the subcutaneous fat tissue was mechanically removed by a scalpel. Afterwards, the skin

pieces were frozen in liquid nitrogen then stored maximally for 12 months at –25°C, until used for the experiment.

Prior to the experiment the skin pieces were allowed to thaw gradually to room temperature and prepared as thin as possible by mechanically removing a part of the dermis. Stratum corneum sheets were isolated by trypsination (Kligman and Christophers, 1964). This was done by spreading the skin sheet with its dermal side on filter paper, which was wetted with 2% aqueous trypsin solution (Pancreas protease; Merck, D-Darmstadt) and incubated for 24 hours at 37°C. After this time the stratum corneum was carefully peeled off from the underlying cells using a blunt forceps. To prevent further enzymatical degradation, the stratum corneum was bathed for several minutes in a 0.01% aqueous solution of trypsin inhibitor (Type II-O: Chicken egg white; Sigma, D-Steinheim), then washed several times in water and subsequently dried and stored at room temperature in a desiccator over silica gel.

In order to prevent any possible changes or degradation of stratum corneum during storage, the isolated sheets were used within 4 months.

3.2.9.2. Permeation experiments through excised human stratum corneum

Permeation experiments were performed in the Franz cell diffusion cells (Fig. 3.1.). The same cell sizes regarding the diffusion area and acceptor volume as well as the same donor and acceptor were used as in the release experiments (3.3.8.). Prior to experiment the stratum corneum sheets were cut into small pieces, (about 1.5 x 1.5 cm), examined for the absence of holes then completely hydrated in water. Before mounting the stratum corneum pieces on the diffusion cells the sheets were placed on polycarbonate filter TMTP, 5 µm (Millipore, D-Eschborn) for higher mechanical stability.

The experiment was carried out at least in triplicate at 37°C. Samples of 250 µl were taken from the acceptor compartment over 29 hours and replaced by fresh buffer. The concentration of hydrocortisone was determined by HPLC as described in 3.2.1.

3.2.10. Pretreatment of excised stratum corneum for DSC and WAXD experiments

In order to obtain sharper transitions and reflections stratum corneum was hydrated to 20% water content by placing it in an desiccator with saturated sodium chloride solution (rel. humidity: 75.2% at room temperature) for 48 hrs. Afterwards, stratum corneum was cut into pieces of about 12 mg which were then immersed in the respective cream bases for 30 minutes at 37°C.

After this time the rests of the cream bases were removed carefully and the stratum corneum sheets were pressed between two filter papers until no fat spots were observed on the filter paper any more.

The stratum corneum sheets were then folded in an aluminium crucible and analyzed by DSC (3.2.2.) or brought in a glass capillary for WAXD measurements (3.2.3.).

3.2.11. Rheological characterization of cream bases by oscillatory measurements

Rheological measurements were performed at 20°C on a controlled stress rheometer CVO/CS (Bohlin Instruments, D-Mühlacker) equipped with a cone and plate system CP4/40 having a cone angle of 4° and a diameter of 40 mm. The split between cone and plate was 150 µm.

Oscillatory measurements were carried out to detect the viscous as well as the elastic properties of the cream bases without disturbing the structure of the base. The measured parameters like phase angle, complex viscosity and the moduli were calculated by software version 5.40.

First, the viscoelastic range for all bases was determined at constant frequency (1Hz) by driving a shear stress ramp. For almost all bases a shear stress of 35 Pa seemed very suitable for the oscillatory measurement within the viscoelastic range except for a few bases (mentioned later) a higher shear stress (100-150 Pa) was chosen as the measured parameters fluctuated strongly at lower shear stress values.

The measurement was repeated three times for each cream base.

3.2.12. Preparation of cream bases

The cream bases were prepared manually according to the instructions of the German Pharmacopoeia, DAB 10. Briefly, the fatty phase was melted at 70°C in a water bath and stirred to room temperature in the case of the anhydrous bases. In the case of hydrous vehicles the aqueous phase was also heated to 70°C on a hot plate and added to the melted fatty phase and then stirred to room temperature. The evaporated water during preparation was replaced after cooling. The prepared cream bases were stored at room temperature and used within maximal two weeks.

3.2.13. Preparation of cream base dilutions

The dilutions of cream bases were performed using an Unguator (GAKO Konietzko GmbH, D-Bamberg). The Unguator (Fig. 3.2.) is defined as a cream base mixer equipped with specialized Unguator-containers. The cream base could be directly prepared in the Unguator even on hot (Alberg, 1998) or used like in this case as a mixer for the already manually prepared bases.

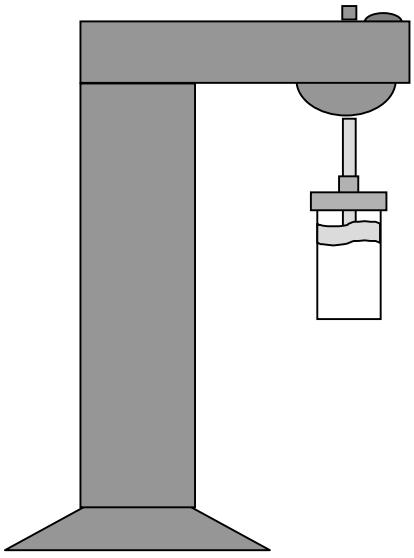


Fig. 3.2. Unguator mixer

The advantage of the Unguator is the complete homogeneity of the diluted preparations even the hydrophilic together with the lipophilic ones. The homogenization was done at room temperature for two minutes at 1000 rpm.

3.2.14. Incorporation of hydrocortisone in the vehicles

Hydrocortisone was incorporated in the already prepared vehicles on cold using the Unguator. The Unguator was proven for its outstanding homogenization of the drug in the base (Zobel et al., 1997). Half of the base was weighed in the Unguator-container, hydrocortisone was added then the container was filled with the rest of the base until the required weight was achieved. The homogenization was done also for 2 minutes at 1000 rpm.

4. Results and discussion

4.1. Cream bases

4.1.1 Composition of cream bases

In order to investigate the effect of diluting a topical semisolid formulation with other cream bases on drug release and permeation through excised human stratum corneum, water-containing hydrophilic ointment DAB (1998) (WHS) with 1% hydrocortisone was chosen as a model cream (WHS 1%). Hydrocortisone was selected as an example for a topically applied corticosteroid because topical corticosteroid preparations are widely diluted in practice.

WHS 1% was diluted with the same base (WHS) and other bases chosen from the German Pharmacopoeia, DAB 1998. The vehicles chosen were considered to cover a wide range of base types regarding the lipophilicity, hydrophilicity and water content. They can be classified into the following types:

- anhydrous hydrophilic base:
  hydrophilic ointment (HS), Hydrophile Salbe DAB 1998
  30% emulsifying cetostearyl alcohol
  35% liquid paraffin
  35% white petrolatum

- anhydrous lipophilic base:
  wool fat ointment (WS), Wollwachsalkoholsalbe DAB 1998
  93.5% white petrolatum
  6.0% wool fat alcohol
  0.5% cetostearyl alcohol

- water-containing hydrophilic bases:

water-containing hydrophilic ointment (WHS), Wasserhaltige Hydrophile Salbe DAB 1998

70% hydrophilic ointment
30% water

non-ionic hydrophilic cream (NHC), Nichtionische Hydrophile Creme DAB 1998

10% cetostearyl alcohol
25% white petrolatum
5% tween 60
10% glycerol 85%
50% water

- water-containing lipophilic base:

water-containing wool fat ointment (WWS), Wasserhaltige Wollwachsalkoholsalbe DAB 1998

50% wool fat ointment
50% water

4.1.2. Preparation of the cream bases

All cream bases were prepared according to the instructions of the German Pharmacopoeia DAB, 1998.

There were two alternatives for the preparation of the dilutions. The first one was the hot-method, i.e. both bases were melted together then stirred to room temperature.

The other possibility was, to stir both bases together at room temperature using the Cito® Unguator (cold-method). In order to select the suitable method for dilution a drug release experiment was carried out. The diluted formulations WHS 1%/HS 1:1, WHS 1%/WHS 1:1 and WHS 1%/WHS 1:2 were prepared by both methods and the amount of hydrocortisone released after 7 hrs was determined.

As seen in Fig. 4.1. the amount of hydrocortisone released from all formulations prepared by the cold-method was greater than those prepared by the hot-method. One interpretation of this finding would suggest that hydrocortisone is partially

degraded when the bases are melted at a temperature of about 70°C. Besides the structure of the diluted formulation prepared by the melting method could be completely different from that prepared by the cold-method. Accordingly, hydrocortisone may be integrated differently in the structure of the base. The hot-method is thus not easily standardized as different bases may need different times for melting.

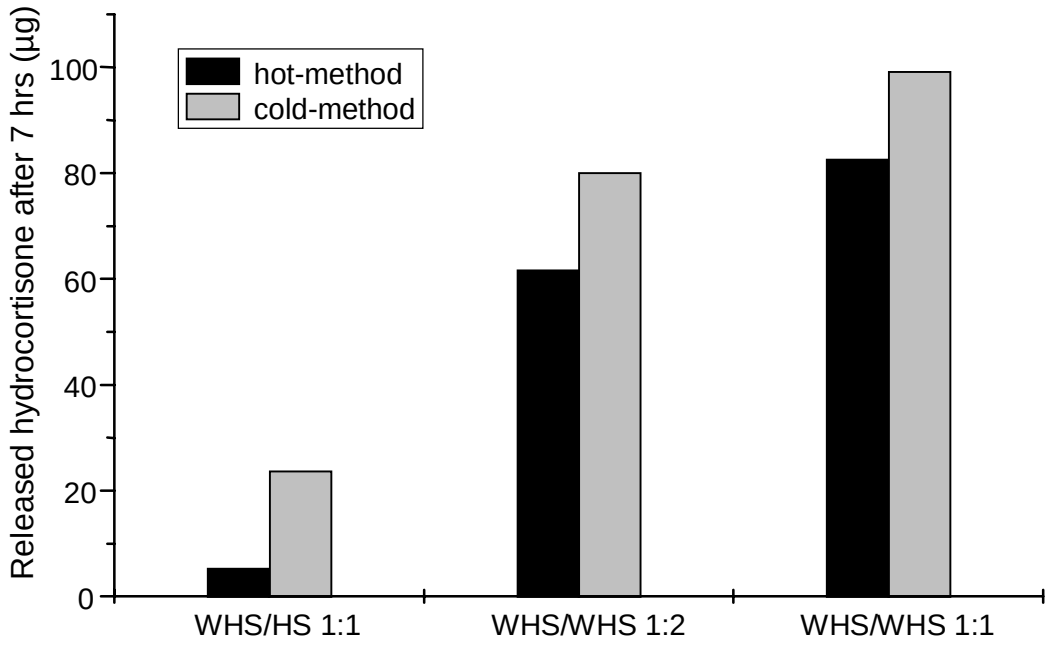


Fig.4.1. Release experiment of hydrocortisone from WHS/HS 1:1, WHS/WHS 1:2 and WHS/WHS 1:1

For the above mentioned reasons and the fact that the dilutions in the practice are mostly done on cold, the cold-method was selected to prepare all diluted formulations in this work. The method is described in detail in 3.2.13.

4.2. Drug release experiments

In order for a drug in a topical formulation to exert its action it must be first released from the preparation to the surface of the skin. Drug release from semisolid preparations is known to be vehicle-dependent (Benninger, 1977). Therefore, one of the most serious incompatibilities that may occur upon diluting a cream preparation with another base is the unexpected change in drug liberation depending on the type of base used for dilution. In this chapter the influence of diluting a cream base with other bases on drug liberation was investigated. Furthermore, the solubility of the drug in the different bases was determined to detect its possible effect on drug release. The drug release experiments and drug solubility in the base were performed as described in 3.2.8. and 3.2.4.2. respectively.

4.2.1. The influence of base type on drug release

As previously mentioned five bases (WHS, WWS, NHC, HS and WS) with different compositions and properties were chosen from the German Pharmacopoeia, DAB 1998 in order to investigate the influence of base type on drug release.
The cream bases were prepared with 1% hydrocortisone and examined for their drug release. The amount of drug released per unit area (µg/cm²) was plotted versus the square root of time ($min^{½}$).

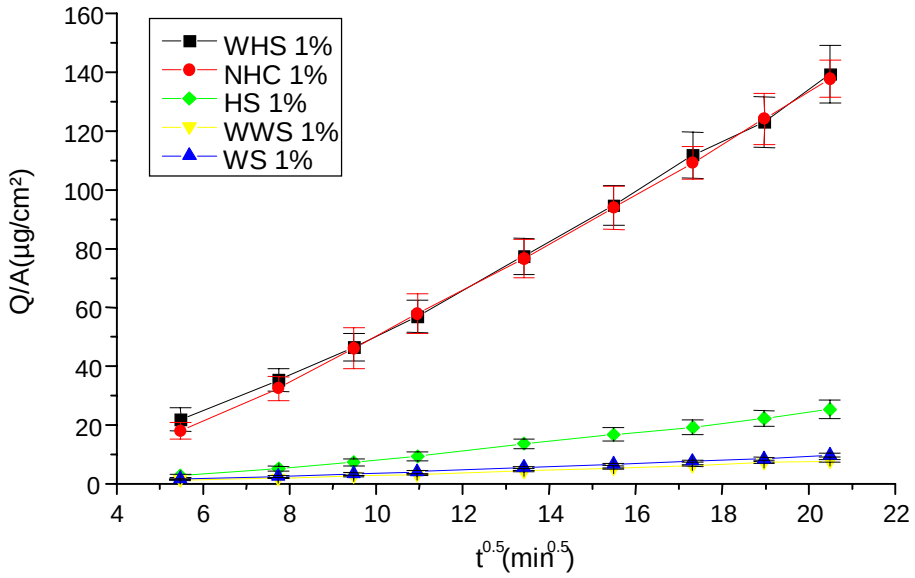


Fig. 4.2. Release of hydrocortisone from 1% WHS, NHC, WWS, HS and WS

Base	Slope [µg/cm²* min$^{-0.5}$]	Liberation coefficient [10^{-7} cm²/s]
WHS 1%	8.63	5.72
NHC 1%	8.33	5.75
HS 1%	1.63	0.60
WWS 1%	0.52	0.14
WS 1%	0.58	0.39

Table 4.1. Liberation coefficients calculated from the slopes of the liberation curves of the studied cream bases

A linear correlationship was obtained between the cumulative amount of drug released in microgram per contact area in square centimetres and the square root of time. Therefore liberation coefficients could be calculated from the slopes of the graphs according to Higuchi equation (2.2.) (Table 4.1.).

Figure 4.2. shows that the liberation rates of WHS (70% water) and NHC (50% water) were very high with no significant difference, followed by HS (anhydrous) with a remarkably low liberation profile. At last WS (anhydrous) and WWS (50% water) showed the lowest liberation rates. This result reveals the great influence of water on drug liberation when incorporated in the hydrophilic bases, while having no effect on liberation when present in lipophilic vehicles.

The solubility of hydrocortisone in all studied formulations was very limited, which means that hydrocortisone was rather suspended in the bases than dissolved, however, only the soluble part of the drug was able to diffuse. Therefore, it was important to determine the saturation concentration (Cs) of hydrocortisone in the different bases in order to reveal its possible influence on drug release (Table 4.2.).

WHS and NHC exhibited the greatest solubilizing capacity for hydrocortisone. The solubility of hydrocortisone is decreased to some extent in HS, while being relatively low in WS. These findings indicate that the solubility of hydrocortisone is significantly reduced in the anhydrous bases. The greater solubility of hydrocortisone in HS compared to WS may be attributed to the presence of emulsifying alcohols in the former which are able to solubilize the drug (Horsch et al., 1973; Loth et al., 1979).

The also noticed very low solubility of the drug in WWS reveals the great solubilizing capacity of the aqueous phase in o/w systems being the external phase whereas playing no role when incorporated inside the base (w/o-systems).
It is obvious that the release of hydrocortisone from the cream bases studied agrees well with the solubility of hydrocortisone in these bases. The release of hydrocortisone thus seems to be dependent on the concentration of dissolved hydrocortisone in the bases.

Base	**Cs [%, w/w]**
WHS	0.012
NHC	0.012
HS	0.005
WWS	0.002
WS	0.0012

Table 4.2. Saturation concentration of hydrocortisone in the different cream bases at 20°C.

Considering the microstructure of the cream bases (Fig. 4.3 a, b, c and d) in addition to the solubility of hydrocortisone helps to explain the different liberation profiles.

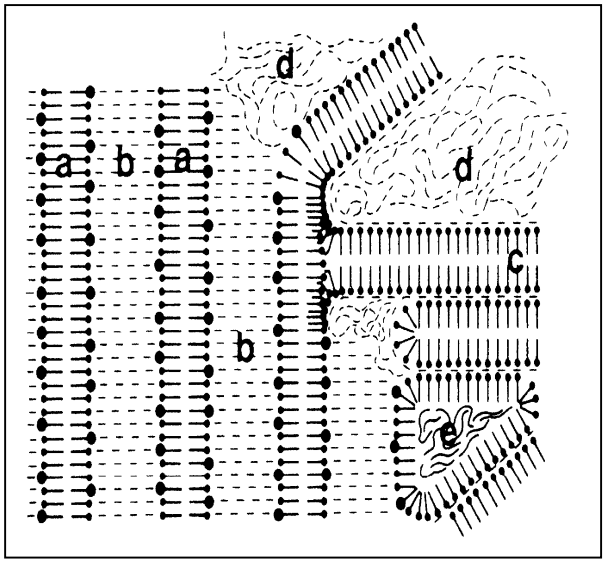


Fig. 4.3.a

a) mixed crystals of cetostearyl alcohol and Na-cetostearyl alcohol sulfate
b) interlamellar fixed water
c) cetostearyl alcohol semihydrate
d) bulk water
e) lipophilic dispersed phase

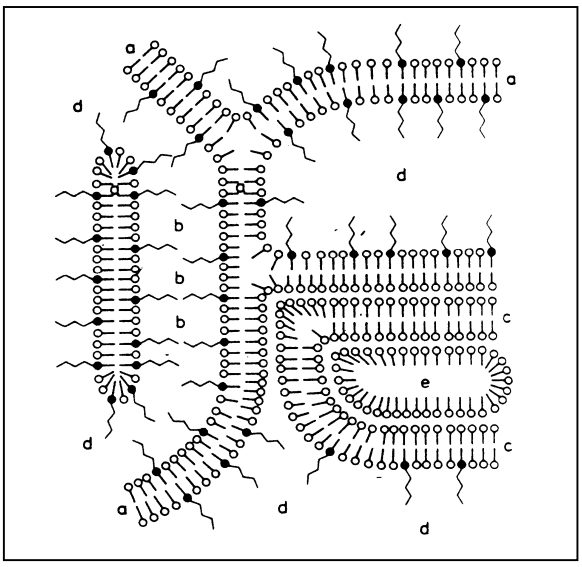


Fig. 4.3.b

a) mixed crystals of non-ionic o/w emulsifier and cetostearyl alcohol
b) interlamellar fixed water
c) lipophilic gel phase
d) bulk water
e) lipophilic dispersed phase

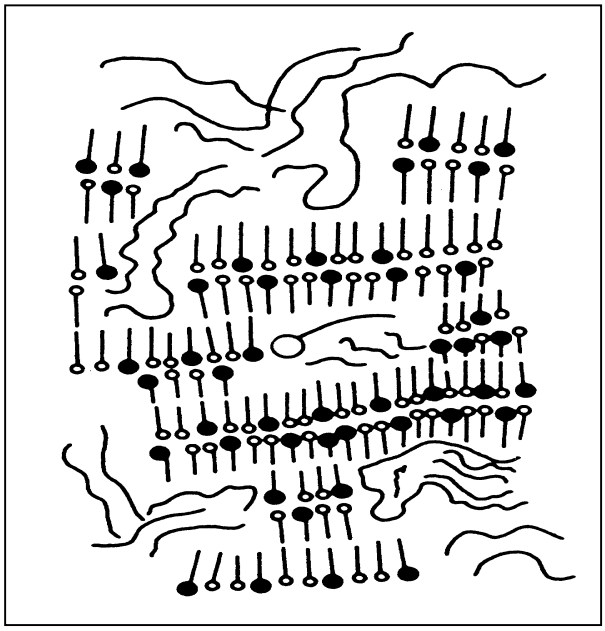


Fig. 4.3.c

Paraffin
Fatty alcohol
Fatty alcohol sulfate

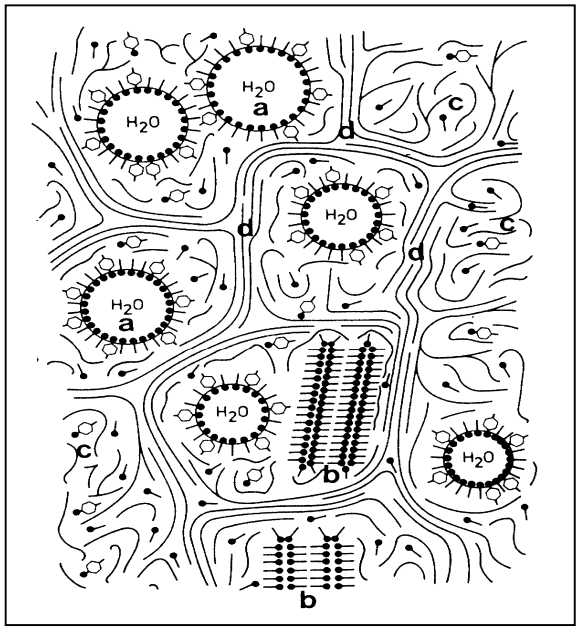


Fig. 4.3.d

a) water drops, b) excess emulsifier crystals
c) liquid lipophilic phase with dissolved emulsifier molecules, d) lipophilic gel phase

Fig. 4.3.a, b, c and d: Microstructure of WHS, NHC, HS and WWS respectively (Niedner and Ziegenmeyer, 1992)

WHS as well as NHC are o/w systems having similar microstructures. They consist of 4 phases, a hydrophilic gel phase, a lipophilic gel phase, an aqueous bulk phase and an internal dispersed lipophilic phase (Niedner and Ziegenmeyer, 1992). The high liberation rates of both creams are probably due to the fast release of hydrocortisone from the external aqueous phase (Benninger, 1977) and the relatively high solubility of hydrocortisone in the bases.

HS is a hydrophilic, anhydrous cream base. Cetostearyl alcohol together with sodium cetostearyl alcohol sulfate build a mixed crystal forming a three-dimensional network in which paraffin and white petrolatum are immobilized (Niedner and Ziegenmeyer, 1992). The different results for WHS and HS in context with both the liberation and solubility studies prove the increased solubility of hydrocortisone in the external aqueous phase.

WS is a lipophilic base consisting mainly of white petrolatum (93.5%), in which the wool fat alcohols are partly suspended and partly dissolved. In this base the wool fat alcohols act as lyotropic solubilizing agents (Loth et al., 1979). The solubility of hydrocortisone in WS is very low, about 10 times lower than that in WHS and NHC and this agrees well with its low liberation rate.

No significant difference between WWS and WS concerning their liberation profiles was observed. WWS is a w/o system, the internal phase is the aqueous phase which is incorporated as droplets inside the system (Niedner and Ziegenmeyer, 1992) having no significant influence on the diffusion and the solubility of hydrocortisone. The diffusion through the external, more viscous, oily phase seems to be the rate limiting step for drug liberation.

Similar results were obtained by Benninger (1977), however, a contradiction is observed concerning the release of the drug from HS in contrast to WS. The author reported that the release from WS was significantly greater than from HS.
This contradiction can be referred to the usage of salicylic acid as a model substance for the liberation study. The author suggested that salicylic acid being not readily soluble in white petrolatum, is mainly concentrated in the mixed crystals of HS thus

only the small part dissolved in white petrolatum is ready to diffuse. In WS, the drug is homogeneously distributed in the base having thereby a greater liberation rate. This finding indicates that hydrocortisone is acting differently being probably homogeneously distributed in both vehicles. Moreover, the greater saturation concentration of hydrocortisone in HS in contrast to WS is considered to be the reason for its greater release from HS.

4.2.2. Influence of dilution on drug liberation

4.2.2.1. Dilution of WHS 1% with different cream bases

4.2.2.1.1. Dilution of WHS 1% with WHS

WHS with 1% hydrocortisone was diluted with WHS in the ratios 1:1, 1:2 and 1:3. As long as the same vehicle was used for the dilution the influence of any variations in structure of the vehicle or in solubility of hydrocortisone is excluded i.e. the dilution resulted only in the reduction of hydrocortisone concentration in the final preparation to the half, third and fourth amount, respectively. Therefore, one would predict a respective decrease in drug liberation.

Fig. 4.4., however, reveals that the dilutions resulted in higher liberation rates than expected. With the dilution ratio of 1:1 the liberation rate was not reduced to the half but it was rather higher. The same was noticed with the ratios of 1:2 and 1:3.
The liberation coefficients were again calculated from the slopes of the graphs and are illustrated in Table 4.3.

These data are in agreement with those of Horsch et al. (1975), who have shown that the increase in liberation is not proportional to the increase of drug concentration, i.e. the four times increase of prednisolone concentration in a suspension vehicle resulted in a raised liberation by the factor of 1.6 only.

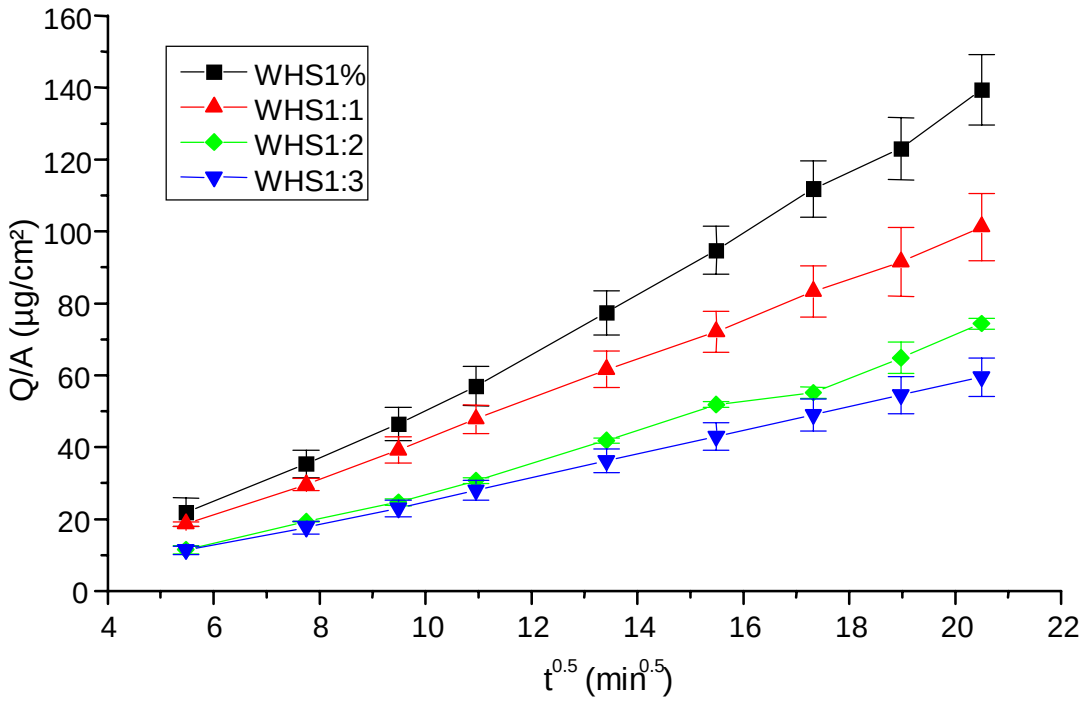


Fig. 4.4. Release of hydrocortisone from 1% WHS diluted 1:1, 1:2 and 1:3 with WHS

Base	Slope [µg/cm²* min$^{-0.5}$]	Liberation coefficient [10^{-7} cm²/s]
WHS 1%	8.63	5.72
WHS 1:1	5.74	5.05
WHS 1:2	4.53	4.76
WHS 1:3	3.29	3.33

Table 4.3. Liberation coefficients calculated from the slopes of the liberation curves of WHS diluted 1:1, 1:2 and 1:3 with WHS

This observation could be explained by regarding the solubility of hydrocortisone in WHS (Table 4.2.). It is clear that in WHS 1% as well as in all diluted formulations the drug is suspended to a great extent, but only the dissolved part of the drug, which remains unchanged in the diluted formulations, can diffuse. In conclusion, the reason behind the reduced drug release in the diluted formulations is not the reduced amount of drug in the bases but probably the variation in concentration gradient between the suspended and the dissolved drug upon dilution. The concentration gradient has a direct influence on the dissolution rate of the suspended particles which supply the base with drug during drug release. The dissolution rate, which is the rate limiting step decreases with increased distance between the suspended particles in the base.

4.2.2.1.2. Dilution of WHS 1% with WWS

It was interesting to investigate the effect of diluting a hydrous hydrophilic base with a hydrous lipophilic one. For this purpose WHS 1% was diluted 1:1, 1:2 and 1:3 with WWS.

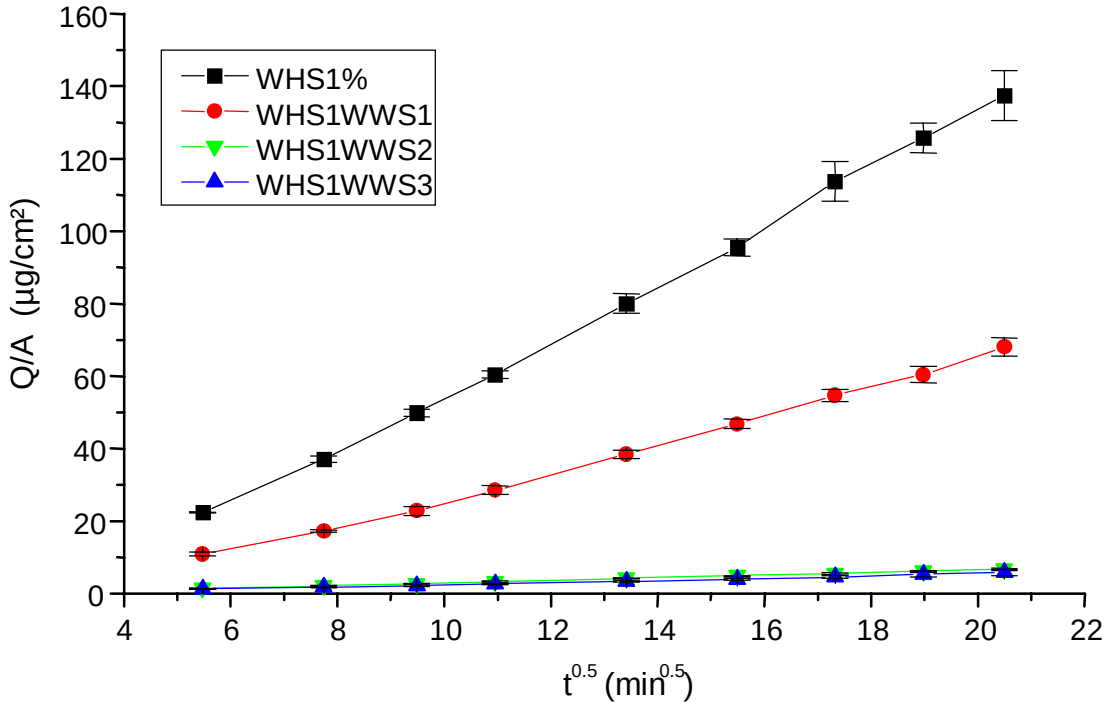


Fig. 4.5. Release of hydrocortisone from WHS 1% diluted 1:1, 1:2 and 1:3 with WWS

Base	Slope [µg/cm²* min$^{-0.5}$]	Liberation coefficient [10^{-7} cm²/s]
WHS 1%	8.63	5.72
WHS/WWS 1:1	4.18	9.29
WHS/WWS 1:2	0.37	0.15
WHS/WWS 1:3	0.38	0.13

Table 4.4. Liberation coefficients calculated from the slopes of the liberation curves of WHS diluted 1:1, 1:2 and 1:3 with WWS

Diluting WHS 1% with WWS shows an interesting liberation profile. The ratio of 1:1 resulted in a reduction in the liberation rate of WHS 1% almost to the half while the dilutions 1:2 and 1:3 decreased the release of hydrocortisone drastically (Fig. 4.5.).

Regarding the solubility of hydrocortisone in the different diluted formulations (Table 4.5) reveals that the solubility of hydrocortisone is greatly reduced upon diluting WHS with WWS in the ratio 1:1 without being significantly affected in further dilutions. Thus the saturation concentration of hydrocortisone in this case only explains the reduced release of hydrocortisone generally upon dilution but does not explain the great difference in hydrocortisone liberation between the 1:1 combination and the other dilutions.

From Table 4.4. it is obvious that the liberation coefficient of the combination 1:1 possesses a remarkably high value although the slope of this formulation is about the half of that of WHS 1%. This contradiction is due to the very low solubility of hydrocortisone in the 1:1 combination in contrast to WHS 1% (Table 4.5). In order to calculate the liberation coefficient the slope² must be divided by the saturation concentration according to Higuchi equation (2.4.).

Base	Cs [%, w/w]
WHS	0.012
WHS/WWS 1:1	0.0033
WHS/WWS 1:2	0.0027
WHS/WWS 1:3	0.0023

Table 4.5. Saturation concentration of hydrocortisone in WHS diluted 1:1, 1:2 and 1:3 with WWS

This finding allows the conclusion that the 1:1 combination is probably still an o/w system, whereas further increasing the amount of WWS in the mixture converts it into a w/o one. This was proven by conductivity and colouring tests. The combination 1:1 showed a blue colouration with methylene blue and a conductivity of 94 µS/cm. The combinations 1:2 and 1:3 gave no blue colour with methylene blue but exhibited a bright red colour with sudan red and a conductivity of just 0.04 µS/cm.

This phenomenon adds a further proof that hydrocortisone is dissolved to a greater extent in the aqueous phase i.e. in o/w systems, where the aqueous phase is the external one allowing the fast release of hydrocortisone whereas in w/o systems the aqueous phase is incorporated inside the base retarding thereby the diffusion of hydrocortisone.

4.2.2.1.3. Dilution of WHS 1% with WS

WS differs from WWS in being anhydrous. How far the absence of water content in WS influences the release of hydrocortisone from the diluted formulations is illustrated in Fig.4.6. and Table 4.6.

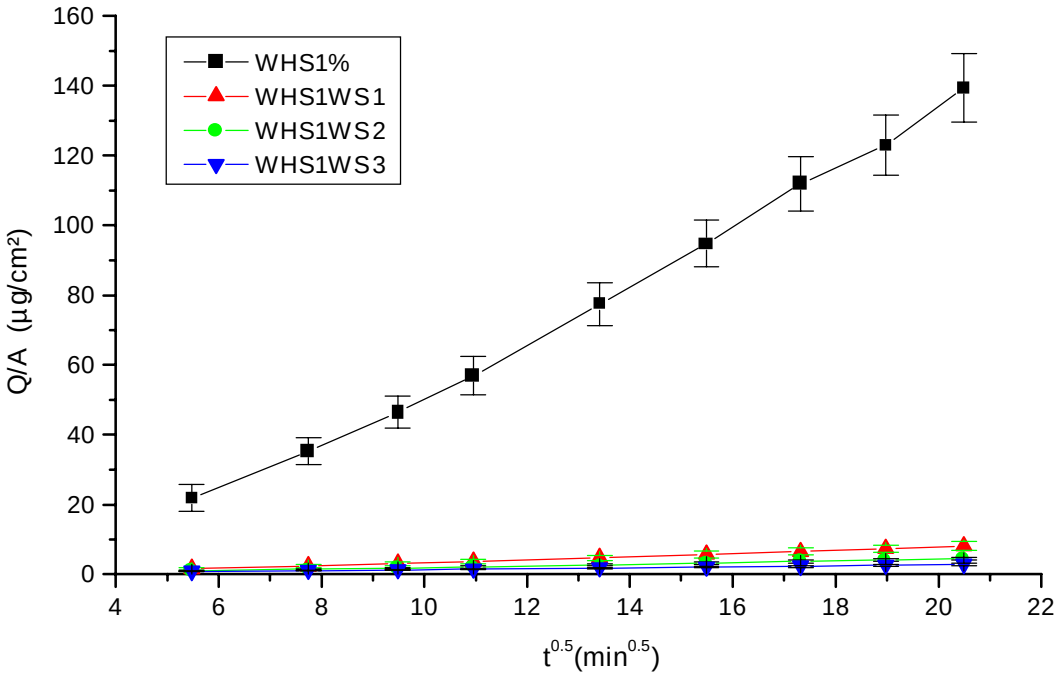


Fig. 4.6. Release of hydrocortisone from WHS 1% diluted 1:1, 1:2 and 1:3 with WS

Diluting WHS 1% with WS resulted in all combinations in very low liberation rates which indicates, that a phase conversion from the w/o system to the o/w system has taken place already with the dilution ratio of 1:1. This was proven with a colouring test using sudan red, which gave a bright red colour for all dilution ratios indicating a lipophilic external phase.

Base	**Slope [µg/cm²* min$^{-0.5}$]**	**Liberation coefficient [10^{-7} cm²/s]**
WHS 1%	8.63	5.72
WHS/WS 1:1	0.48	0.23
WHS/WS 1:2	0.37	0.15
WHS/WS 1:3	0.38	0.13

Table 4.6. Liberation coefficients calculated from the slopes of the liberation curves of WHS diluted 1:1, 1:2 and 1:3 with WS

It is worthy to note that the liberation coefficients of all dilutions with WS as well as WWS 1% and the combinations WHS/WWS 1:2 and 1:3 have similar values which are all significantly lower than WS 1% (Tables 4.1, 4.4. and 4.6). This finding allows the conclusion that, all these formulations being w/o systems, permit only the part of the drug which is soluble in the outer fatty base to diffuse whereas the release of hydrocortisone which is present in the internal aqueous phase is retarded.

The saturation concentration of hydrocortisone is greatly reduced upon dilution as seen in Table 4.7. Moreover, the 1:2 and 1:3 combinations have identical values being not significantly different from the 1:1 dilution. These data correlate well with the release of hydrocortisone from the bases.

Base	**Cs [%, w/w]**
WHS	0.012
WHS/WS 1:1	0.002
WHS/WS 1:2	0.0015
WHS/WS 1:3	0.0015

Table 4.7. Saturation concentration of hydrocortisone in WHS diluted 1:1, 1:2 and 1:3 with WS

4.2.2.1.4. Dilution of WHS 1% with HS

Diluting WHS 1% with HS which has the same composition as WHS, with exception of being anhydrous, not only reduced the hydrocortisone concentration but it also decreased the water content. As previously shown, the decrease in hydrocortisone

concentration does not have a major influence on drug release as long as the drug is suspended in the vehicle. Thus the factor that is expected to affect drug release is the amount of the aqueous phase in addition to the type of the system being an o/w system or a w/o one. To what extent drug liberation from WHS is influenced by the dilution with HS is demonstrated in Fig. 4.7. and Table 4.8.

From Fig. 4.7. it is obvious that the hydrocortisone liberation was reduced drastically upon dilution for all formulations. However, the release rate from the dilution 1:1 was greater than the dilutions 1:2 and 1:3 which were not significantly different. Colouring and conductivity tests were carried out to identify the type of emulsions of the resulted dilutions. Both tests showed that the combination 1:1 is still an o/w system, whereas a phase conversion has taken place for the other two dilutions. This finding explains the greater liberation coefficient of the 1:1 formulation (Table 4.8.) in contrast to the others.

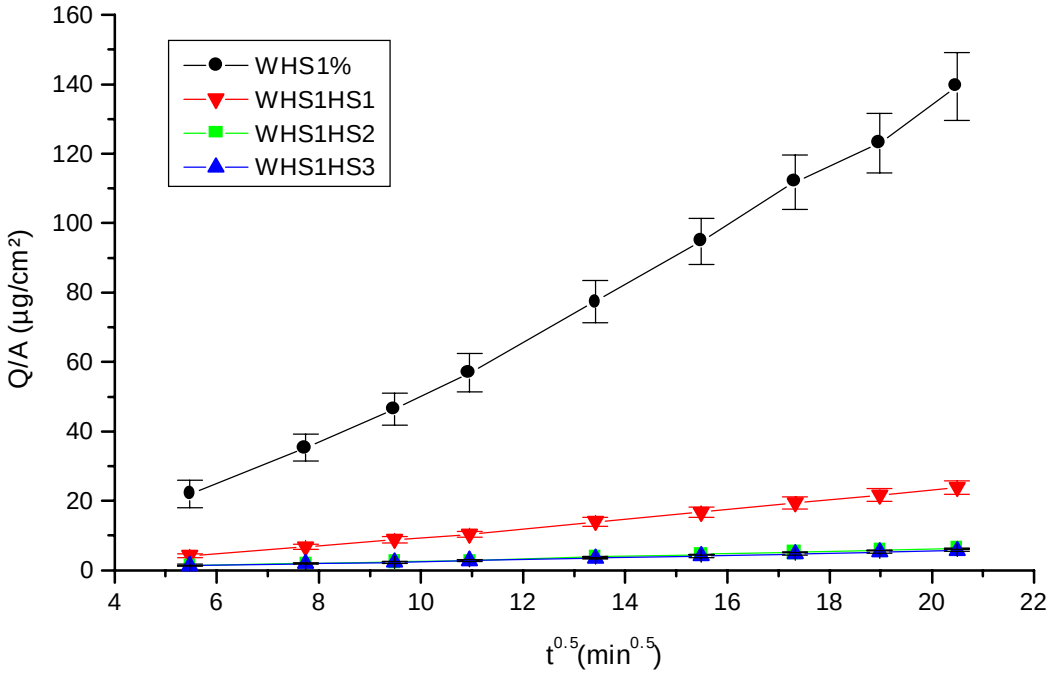


Fig. 4.7. Release of hydrocortisone from WHS 1% diluted 1:1, 1:2 and 1:3 with HS

Base	Slope [µg/cm²* min$^{-0.5}$]	Liberation coefficient [10^{-7} cm²/s]
WHS 1%	8.63	5.72
WHS/HS 1:1	1.43	0.63
WHS/HS 1:2	0.35	0.08
WHS/HS 1:3	0.33	0.09

Table 4.8. Liberation coefficients calculated from the slopes of the liberation curves of WHS diluted 1:1, 1:2 and 1:3 with HS

Nevertheless, the 1:1 dilution, despite of being an o/w system, revealed a significantly low liberation profile compared to WHS 1% which confirms again that hydrocortisone is dissolved to a great extent in the external aqueous phase. Decreasing the amount of aqueous phase by dilution with HS therefore affects the drug release negatively.

Base	Cs [%, w/w]
WHS	0.012
WHS/HS 1:1	0.0066
WHS/HS 1:2	0.005
WHS/HS 1:3	0.005

Table 4.9. Saturation concentration of hydrocortisone in WHS diluted 1:1, 1:2 and 1:3 with HS

Regarding Table 4.9. it is obvious that the solubility of hydrocortisone in the formulations – being slightly greater in the 1:1 combination than in the other two dilutions which have identical values - is in agreement with the above mentioned conclusion.

However, it seems surprising that HS 1% being anhydrous thus possessing a lower solubilizing capacity for hydrocortisone has almost the same liberation coefficient as WHS/HS 1:1 which is an o/w system with greater saturation concentration of hydrocortisone. An explanation for this finding could be that the decreased concentration of the drug, and consequently the decreased concentration gradient between suspended and dissolved drug, in the diluted formulation (WHS/HS 1:1) is compensated by the increase in hydrocortisone solubility in the base.

4.2.2.1.5. Dilution of WHS 1% with NHC

It was interesting to investigate the effect of using a hydrous hydrophilic base other than WHS as a diluting vehicle. For this purpose the non-ionic hydrophilic cream, NHC, was chosen.

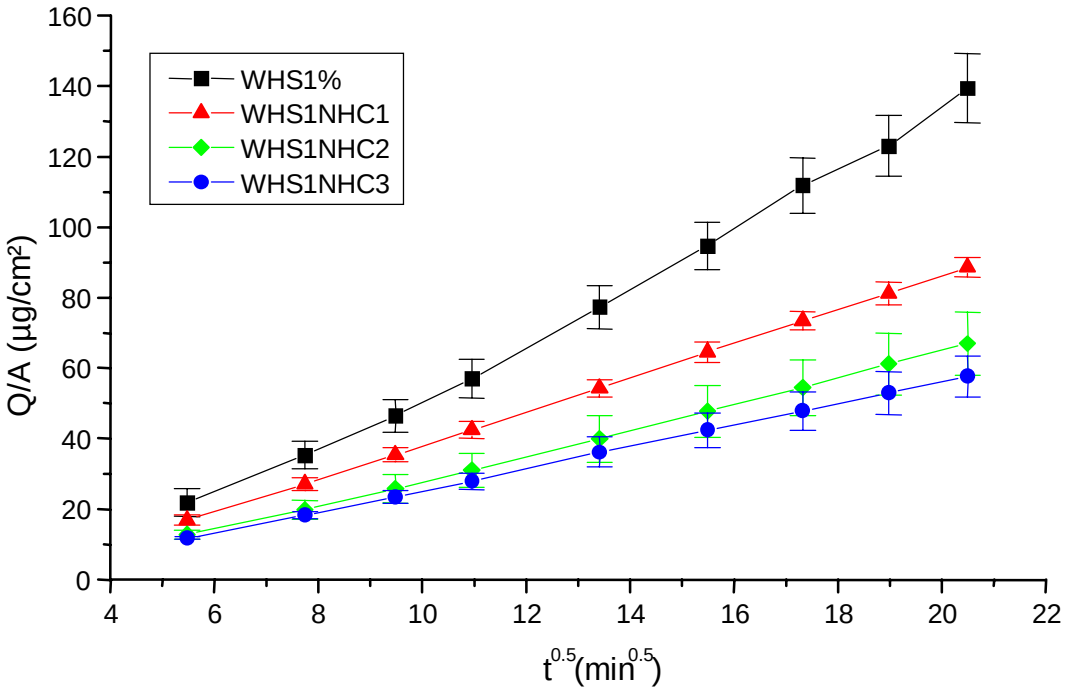


Fig. 4.8. Release of hydrocortisone from 1% WHS diluted 1:1, 1:2 and 1:3 with NHC

From Fig. 4.8. it is to be noticed that the 1:1 dilution reduced the release of hydrocortisone only to some extent being only slightly greater than further dilutions (1:2 and 1:3) which have very close liberation rates. The relative high liberation rates of all formulations is referred to their base type being all o/w systems with high water content; therefore, hydrocortisone readily diffuses from the external aqueous phase.

Furthermore, the relative good solubility of hydrocortisone in all formulations as shown in Table 4.11 can be considered as a further explanation for the high liberation profiles. It is believed that a good solubility of the drug in the vehicle is associated with a fast release, resulting in a steep concentration gradient in the base with a great difference between saturation concentration and the concentration of the drug at the interface through which the molecules continue to diffuse (Loth et al., 1984).

The reduced liberation rates of the dilutions, despite of having all the same amount of dissolved drug which is equal to that of WHS 1% (Table 4.11.), can only be interpreted as the variation in concentration gradient between suspended and dissolved drug upon dilution.

Base	**Slope** **[µg/cm²* min$^{-0.5}$]**	**Liberation coefficient** **[10^{-7} cm²/s]**
WHS 1%	8.63	5.72
WHS/NHC 1:1	4.86	3.82
WHS/NHC 1:2	3.82	2.99
WHS/NHC 1:3	3.02	2.44

Table 4.10. Liberation coefficients calculated from the slopes of the liberation curves of WHS diluted 1:1, 1:2 and 1:3 with NHC

It is obvious that diluting WHS 1% with NHC gave similar liberation profiles like those obtained when WHS 1% was diluted with WHS (Fig. 4.4.). This was expected as both are o/w systems having a similar four-phase microstructure. Moreover, both bases have identical dissolving capacity for hydrocortisone as shown in Table 4.11.

Base	**Cs [%, w/w]**
WHS	0.012
WHS/NHC 1:1	0.012
WHS/NHC 1:2	0.012
WHS/NHC 1:3	0.012

Table 4.11. Saturation concentration of hydrocortisone in WHS diluted 1:1, 1:2 and 1:3 with NHC

4.2.2.1.6. Dilution of WHS 1% with white petrolatum

It seemed important to investigate the effect of diluting WHS 1% with white petrolatum being a main component of all studied bases. White petrolatum is lipophilic in nature containing no emulsifying components.

From Fig. 4.9. it is obvious that the liberation rate was reduced drastically as a result of the phase conversion upon dilution. This finding matches with the results obtained with WHS 1% diluted with other lipophilic bases.

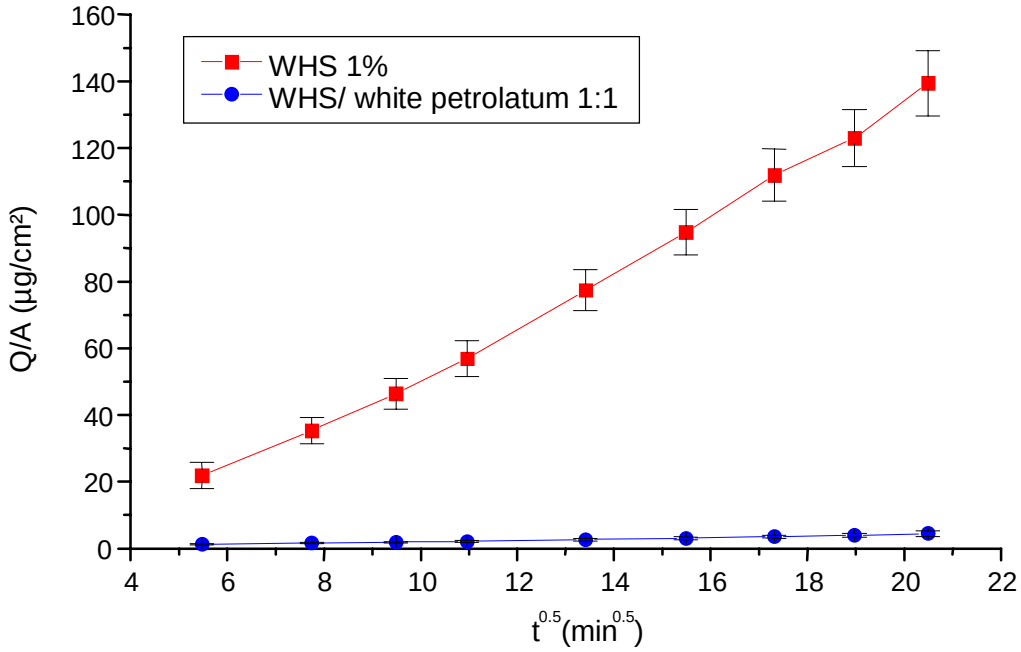


Fig. 4.9. Release of hydrocortisone from WHS 1% diluted 1:1 with white petrolatum

In order to investigate the effect of adding hydrophilic and lipophilic emulsifiers to white petrolatum on liberation the release rates of hydrocortisone from the formulations WHS/HS 1:1 and WHS/WS 1:1 respectively were compared to that of WHS/white petrolatum 1:1.

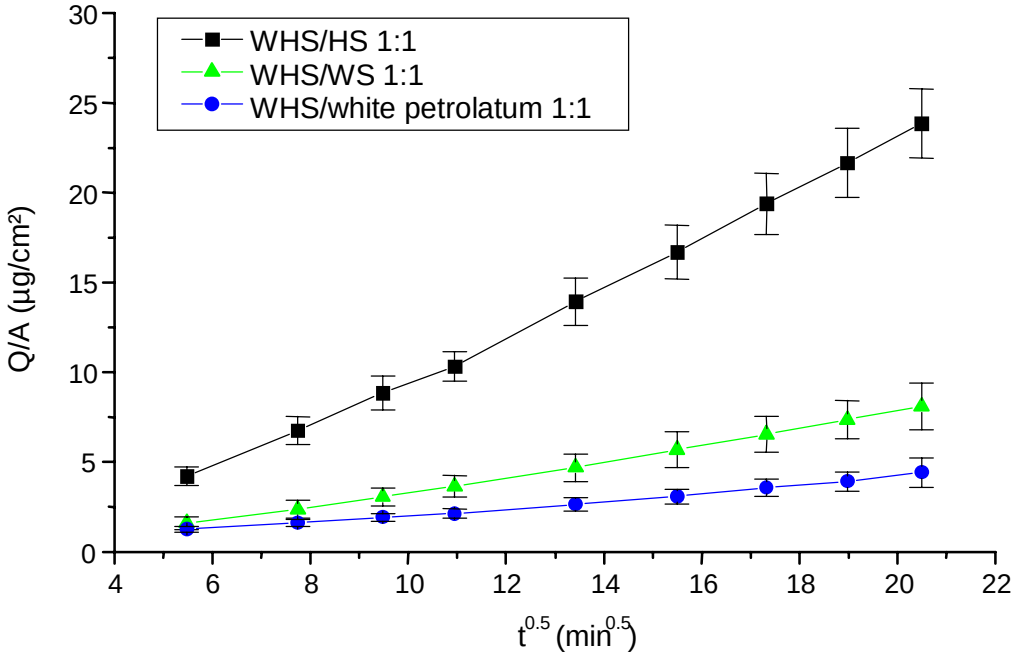


Fig. 4.10. Release of hydrocortisone from WHS 1% diluted 1:1 with HS, WS and white petrolatum

Fig 4.10. shows that the formulation WHS/HS 1:1 exhibited the greatest liberation profile among the three preparations. It was shown previously (4.2.2.1.2.) that this combination is an o/w system, i.e. the emulsifier maintained the hydrophilic character of the base. However, the emulsifier is not the only factor influencing the drug release in this case, as the water content plays also a major role. The presence of 35% water content in the formulation allowed the hydrophilic emulsifier to retain the hydrophilic character of the base. In the case of lower water contents a phase conversion to the w/o type takes place in spite of the presence of the hydrophilic emulsifier. Furthermore, the emulsifier possesses a solubilizing effect, thus HS has the greatest solubilizing capacity for hydrocortisone as shown in Table 4.12. It is interesting to note that the liberation coefficient as well as the drug solubility of WHS/HS 1:1 is about three times greater than those of WHS/WS 1:1 (Table 4.12 and 4.13).

Base	**Cs [%, w/w]**
WHS	0.012
WHS/HS 1:1	0.0066
WHS/WS 1:1	0.002
WHS/white petrolatum 1:1	0.0015

Table 4.12. Saturation concentration of hydrocortisone in WHS diluted 1: 1with HS, WS and white petrolatum

The liberation profiles of the formulations WHS/WS 1:1 and WHS/white petrolatum 1:1 were very close i.e. the addition of a lipophilic emulsifier to white petrolatum did not effectively alter the drug release as seen in Fig. 4.10. However, the increased hydrocortisone release from WHS/WS 1:1 in contrast to WHS/white petrolatum 1:1 is suggested to be attributed to the solubilizing effect of the emulsifier to hydrocortisone (Table 4.13.).

Base	Slope [µg/cm²* min$^{-0.5}$]	Liberation coefficient [10^{-7} cm²/s]
WHS 1%	8.63	5.72
WHS/HS 1:1	1.43	0.63
WHS/WS 1:1	0.48	0.23
WHS/white petrolatum 1:1	0.21	0.055

Table 4.13. Liberation coefficients calculated from the slopes of the liberation curves of WHS diluted 1:1with HS, WS and white petrolatum

4.2.2.2. Dilution of WWS 1% with different cream bases

It seemed also interesting to incorporate the drug into a lipophilic base and to investigate the effect on drug release when diluted with the other cream bases. WWS 1% was chosen for this purpose as a model cream. It was diluted in the ratio 1:1 with WHS, HS and WWS.

Fig. 4.11. reveals that diluting WWS 1% with WHS resulted in a remarkable increase in drug release, in spite of reducing the amount of drug to the half. This interesting phenomenon is of course due to the phase conversion to the o/w system upon dilution, permitting thereby hydrocortisone to diffuse fast from the external aqueous phase.

Using HS for the dilution showed only a minimal increase in liberation, whereas diluting WWS 1% with the same base reduced the liberation more than to the half. The slight increase in liberation upon dilution with HS is referred to the increased amount of dissolved drug due to the solubilizing effect of the emulsifying mixed crystals in HS.

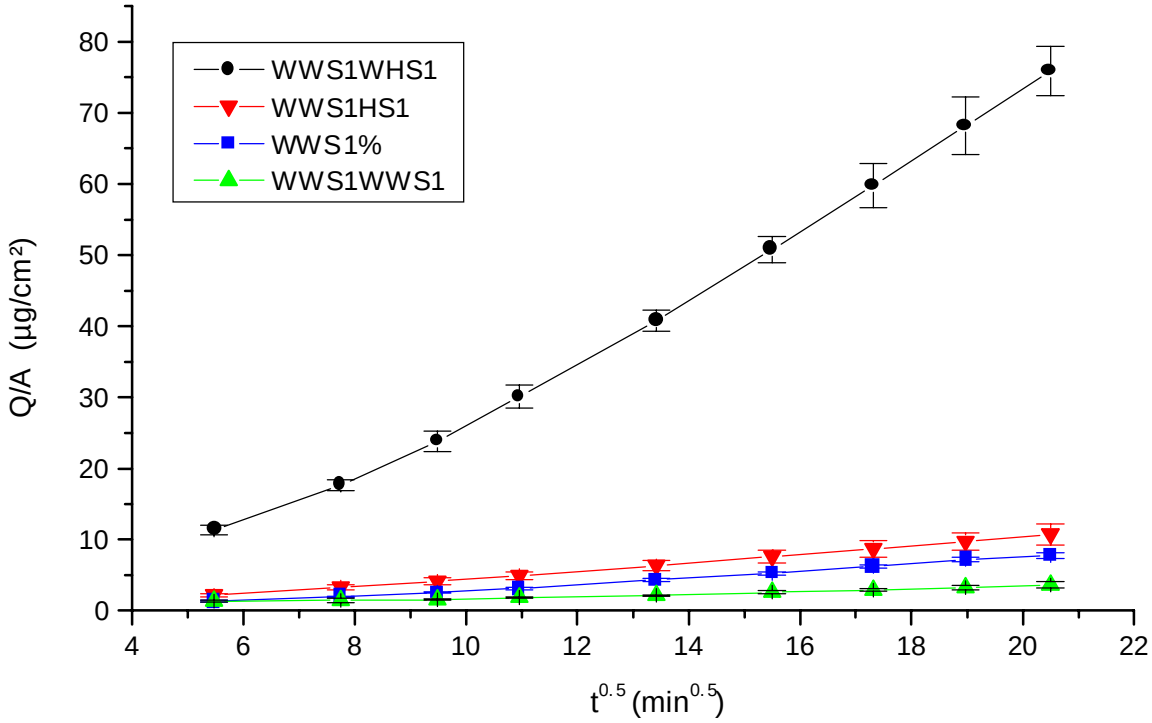


Fig. 4.11. Release of hydrocortisone from WWS 1% diluted 1:1 with WHS, HS and WWS

Base	Slope [µg/cm²* min$^{-0.5}$]	Liberation coefficient [10^{-7} cm²/s]
WWS 1%	0.52	0.14
WWS/WHS 1:1	4.4	10.27
WWS/HS 1:1	0.57	0.194
WWS/WWS 1:1	0.16	0.022

Table 4.14. Liberation coefficients calculated from the slopes of the liberation curves of WHS diluted 1:1 with HS, WS and white petrolatum

The decreased release noticed for the formulation WWS/WWS 1:1 can be only attributed to the reduced concentration gradient between suspended and dissolved drug.

Base	Cs [%, w/w]
WWS	0.002
WWS/WHS 1:1	0.0033
WWS/HS 1:1	0.003
WWS/WWS 1:1	0.002

Table 4.15. Saturation concentration of hydrocortisone in WHS diluted 1:1 with HS, WS and white petrolatum

Comparing the systems WHS 1% diluted with WWS 1:1 (Fig. 4.5.) and WWS 1% diluted with WHS 1:1 (Fig. 4.11.) revealed no significant difference in their liberation profiles (Fig.4.12.). In conclusion, incorporating the drug in either of the two bases before dilution does not affect drug release from the final formulation i.e. the distribution of the drug between the lipophilic and hydrophilic phases in the diluted preparation is independent of the order of dilution.

In order to confirm this finding the hydrocortisone liberation rate of NHC 1% diluted with WHS 1:1 was compared with that of the previously investigated system WHS 1%/NHC 1:1 (Fig. 4.8.). Fig. 4.13. reveals that both systems liberate the drug identically, proving thereby the conclusion mentioned above.

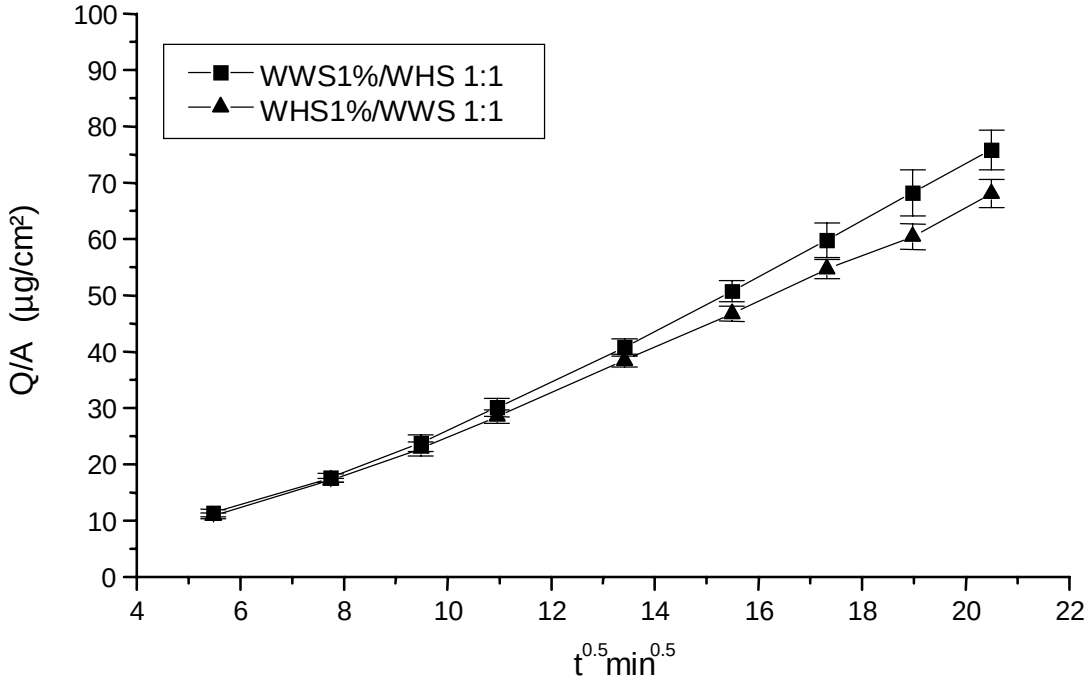


Fig. 4.12. Release of hydrocortisone from WHS1% diluted with WWS 1:1 and from WWS 1% diluted with WHS 1:1

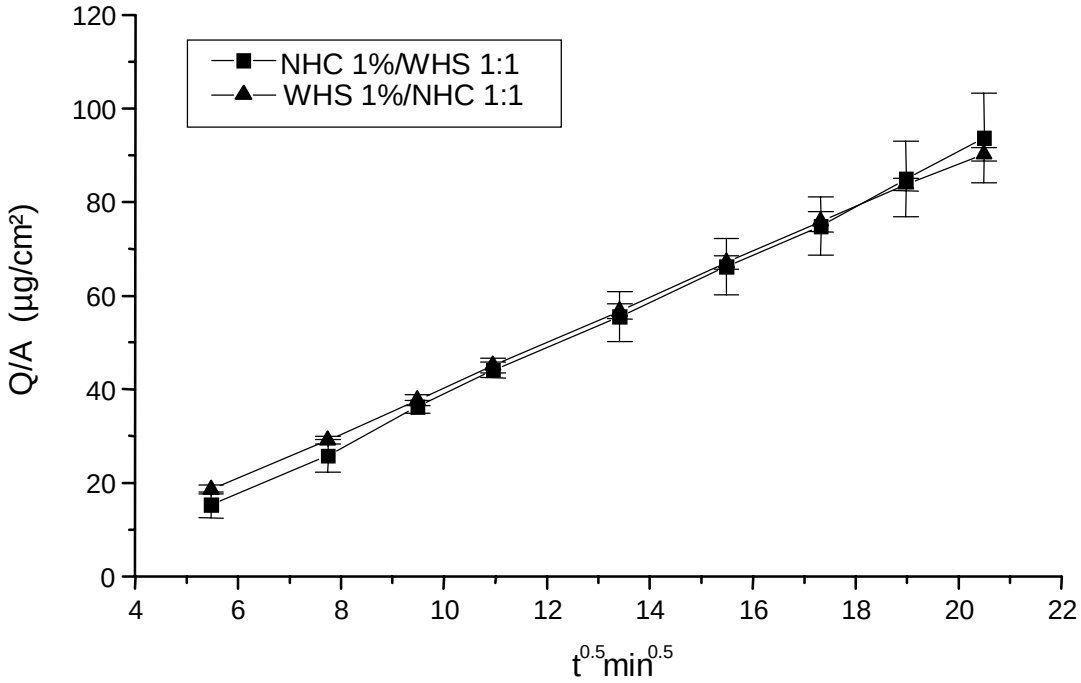


Fig. 4.13. Release of hydrocortisone from WHS1% diluted with NHC 1:1 and from NHC 1% diluted with WHS 1:1

4.2.3. The influence of water content on drug release

Water content is known to influence drug release from semisolid preparations (Niemi et al., 1989; Junginger, 1991; Preiss, 1993). In this study, regarding the different undiluted as well as diluted preparations, it was noticed that water content only affects drug release when incorporated in hydrophilic bases i.e. constituting the external phase, while having no effect when present in lipophilic vehicles.

In order to reveal the effect of the stepwise reduction of water content in a hydrophilic base on hydrocortisone liberation, the water content in WHS, which is 70% was reduced to 50%, 30% and 15%. The obtained formulations were prepared with 1% hydrocortisone and tested for drug release. The results are illustrated in Fig. 4.14.

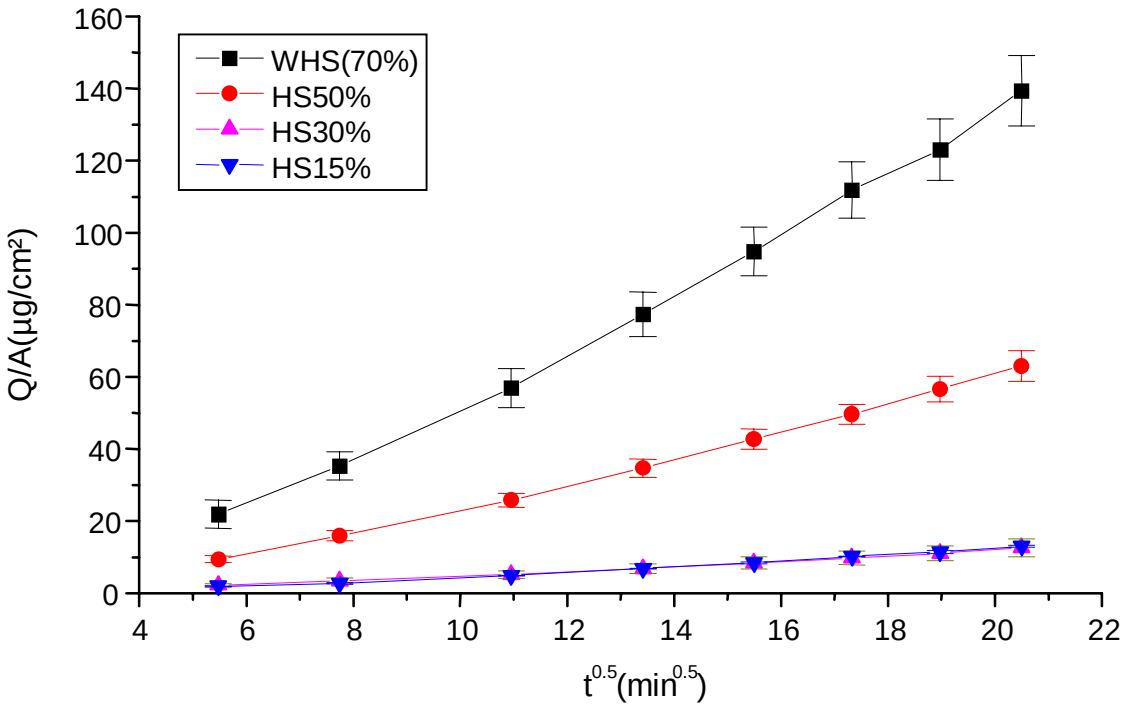


Fig. 4.14. Release of hydrocortisone from 1% WHS (70% water content), 1% HS (50%), 1% HS (30%) and 1% HS (15%)

Fig. 4.14. shows that 20% reduction of water content (from 70%-50%) reduced the drug release more than to the half taking in consideration that it is still an o/w system (Table 4.16.). This reduction of the water content reduced on the one hand the amount of dissolved drug in the base (Table 4.17.) and decreased on the other hand the amount of external aqueous phase (bulk water) in which hydrocortisone is free for release.

HS 30% and HS 15% showed very low liberation rates without being significantly different. Colouring and conductivity tests ensured a phase conversion in these formulations; thus hydrocortisone dissolved in the aqueous phase became fixed in the inside of the base. It is interesting to note that the release of hydrocortisone from WHS/HS 1:1 (35% water), which is still an o/w system (4.2.2.1.4) was found to be significantly higher than from the formulations HS 30% and HS 15% (Table 4.16 and Fig. 4.15.). This indicates that 5% reduction in water content (from 35%-30%) was enough to convert the phase. After phase conversion any further reduction in water content does not influence drug release.

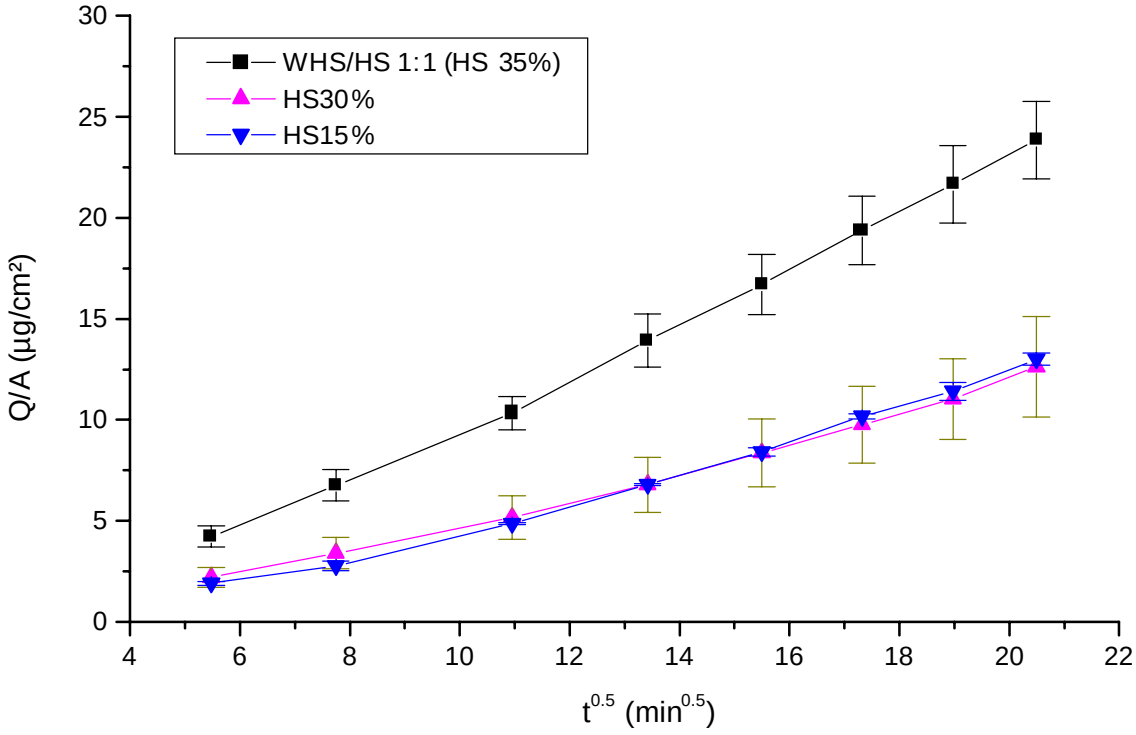


Fig. 4.15. Release of hydrocortisone from WHS 1%/HS 1:1 (35% water), 1% HS (30%) and 1% HS (15%),

Base	Slope [µg/cm²* min$^{-0.5}$]	Liberation coefficient [10^{-7} cm²/s]
1% WHS	8.63	5.72
1% HS (50%)	3.58	1.28
WHS1% /HS 1:1 (35%)	1.43	0.63
1% HS (30%) & 1% HS (15%)	0.75	0.133

Table 4.16. Liberation coefficients calculated from the slopes of the liberation curves of 1% WHS, 1% HS (50%), WHS1% /HS 1:1 (35% water), 1% HS (30%) and 1% HS (15%)

Base	Cs [%, w/w]
WHS	0.012
HS (50%)	0.009
WHS/HS 1:1 (35%)	0.0066
HS (30%) & HS (15%)	0.005

Table 4.17. Saturation concentration of hydrocortisone in WHS, WHS/HS 1:1 (35% water), HS (30%) and HS (15%)

4.2.4. The influence of emulsifier on drug release

It is reported in the previous chapter (4.2.3) that 20% reduction of water content (from 70%-50%) in a hydrophilic base decreased the drug release more than to the half. Taking in consideration that NHC and WHS have a similar microstructure (4.2.1.) and the same dissolving capacity for hydrocortisone (Table 4.2.) it is surprising that NHC despite of having only 50% water possesses the same release rate for hydrocortisone as WHS with 70% water content (4.2.1.). However, regarding the composition of both bases it is noticed that they contain different types and amounts of emulsifiers. WHS contains 9% emulsifying cetostearyl alcohol which is a mixture of cetostearyl alcohol and sodium cetostearyl sulfate being an anionic emulsifier, whereas NHC contains 10% cetostearyl alcohol in addition to 5% tween 60 being both non-ionic emulsifiers.

In order to reveal the influence of emulsifier type on drug liberation the water content in WHS was reduced to 50% to be equal to that of NHC, simultaneously the amount of emulsifier in NHC was decreased to 9% (6% cetostearyl alcohol and 3% tween 60), the remaining 6% were replaced by white petrolatum. The resulting formulations WHS and NHC (water 50% and emulsifier 9%) both containing 1% hydrocortisone were tested for drug release.

From Fig. 4.16. it is obvious that both bases in spite of having the same amounts of water and emulsifier differ greatly in their liberation profiles for hydrocortisone. NHC (9% emulsifier) exhibits a significant greater liberation coefficient than WHS (50%). (Table 4.18.). This finding could be explained by regarding the drug solubility in both bases. Table 4.19. reveals the greater solubilizing capacity of the non-ionic surfactant in NHC for hydrocortisone than does the ionic surfactant in WHS having both the same water content. This result reveals the pronounced effect of emulsifier type on drug solubility and subsequently on drug release.

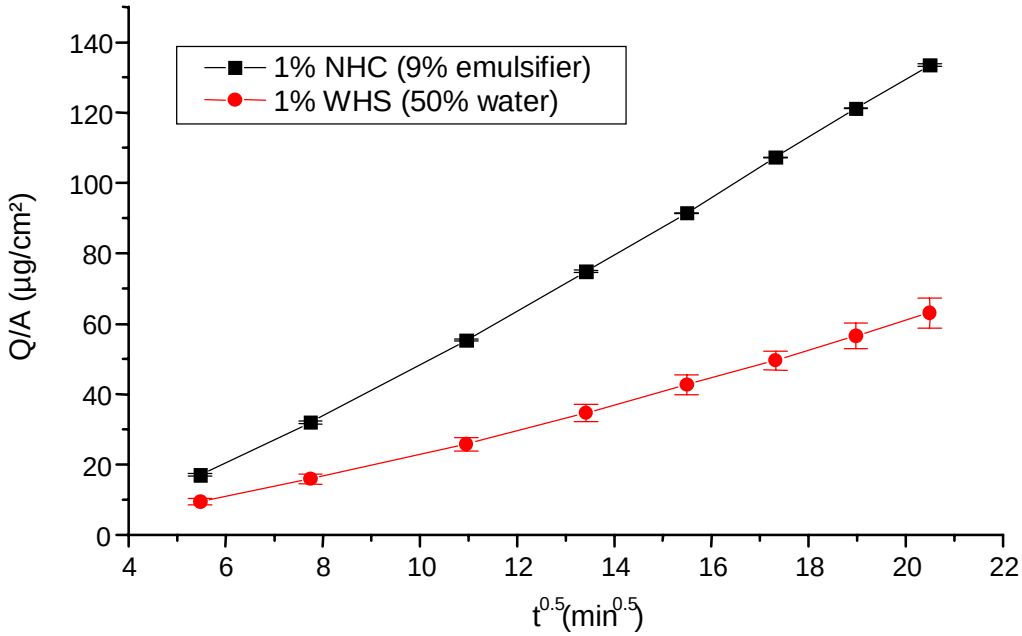


Fig. 4.16. Release of hydrocortisone from 1% NHC and 1% WHS both containing 50% water content and 9% emulsifier

Moreover, comparing the release of hydrocortisone from the formulations NHC with 9% and 15% emulsifier as well as its solubility in these bases no difference could be detected as shown in Fig. 4.17. and Table 4.19 respectively revealing thereby the negligible effect of the emulsifier concentration change on hydrocortisone liberation.

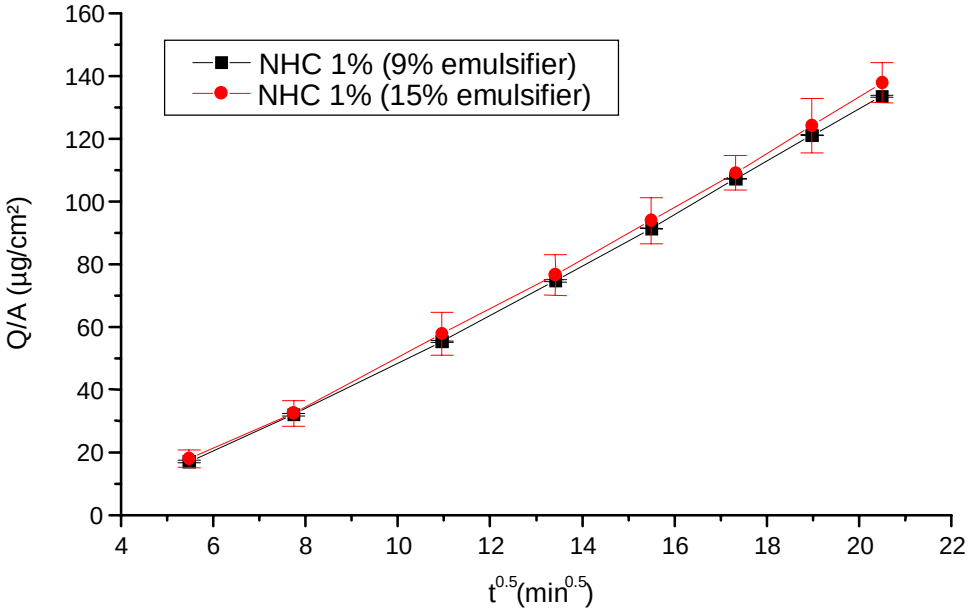


Fig. 4.17. Release of hydrocortisone from 1% NHC (9% emulsifier) and 1% NHC (15% emulsifier)

Similar results were obtained by Turakka and Toikkanen (1983). The authors reported that maximum steady state release of the drug occurred at surfactant concentrations of 0.2 to 5%, above that e.g. at concentrations of 10-15% the release rate was only slightly influenced.

In order to confirm the effect of emulsifier type on the release of hydrocortisone away from the influence of water content, the systems NHC anhydrous and HS (WHS anhydrous) were compared with regard to drug solubility and liberation.

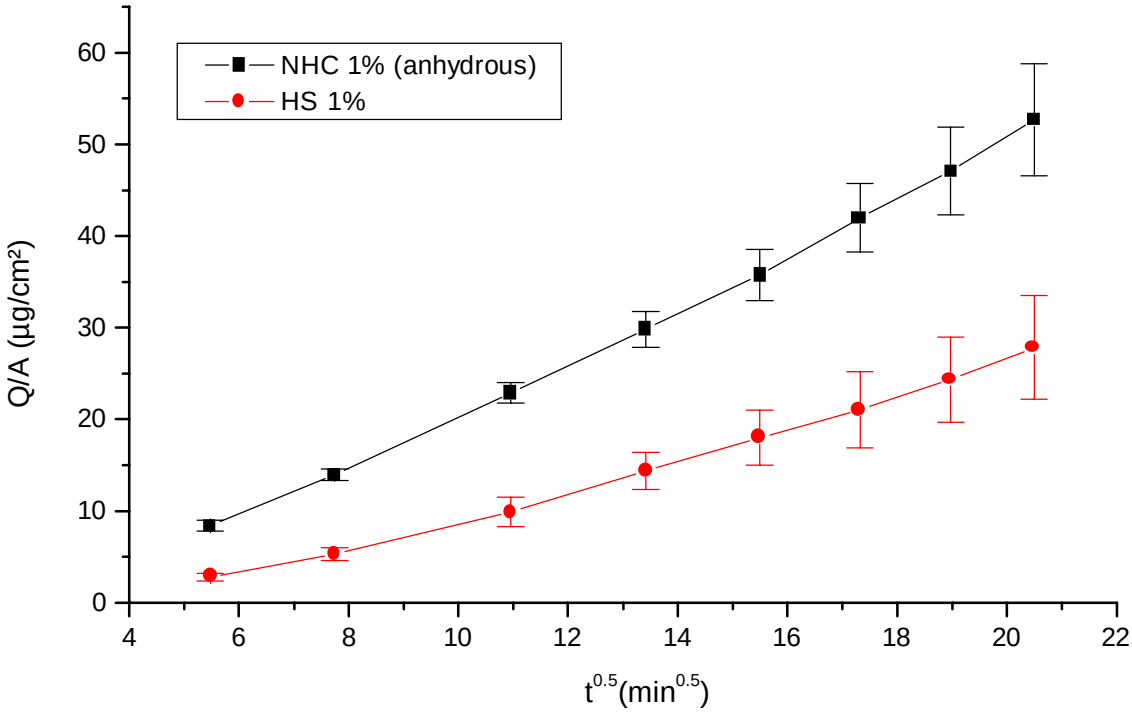


Fig. 4.18. Release of hydrocortisone from anhydrous 1% NHC and 1% HS

Fig. 4.18. reveals a significant greater release of hydrocortisone from 1% NHC anhydrous over 1% HS. This observation is attributed to the pronounced higher solubility of hydrocortisone in NHC anhydrous compared to HS (Table 4.19.) which was surprisingly identical to its solubility in NHC (50% water content). It is to be noted that this effect is opposing that observed for hydrocortisone solubility in WHS, which was greatly influenced by the amount of aqueous phase.

This finding allows the conclusion that the water content in vehicles emulsified by non-ionic emulsifiers does not influence drug solubility as does in bases containing ionic emulsifiers. These data are in agreement with those of Niemi et al. (1989) who have reported that the extent of effect of water content on release of hydrocortisone in the presence of ionic surfactants being more pronounced than that of the non-ionic ones. Taken together, these results suggest that the water content is not of special importance in creams containing an emulsifier that does not significantly increase the amount of hydrocortisone dissolved in the water phase (Niemi et al., 1989).

However, 1% NHC anhydrous shows significant lower liberation profile than NHC 1% (50% water content) in spite of having same capacity for hydrocortisone. This effect is probably referred to other factors playing also an important role on drug release e.g. viscosity.

Base 1%	**Slope** **[µg/cm²* min$^{-0.5}$]**	**Liberation coefficient** **[10^{-7} cm²/s]**
WHS (50%)	3.58	1.28
NHC (9% emulsifier)	8.23	5.04
NHC	8.33	5.75
NHC (anhydrous)	2.94	0.638
HS	1.63	0.60

Table 4.18. Liberation coefficients calculated from the slopes of the liberation curves of 1% WHS (50% water), 1% NHC (9% emulsifier), 1% NHC, 1% anhydrous NHC and 1% HS

Base	Cs [%, w/w]
WHS (50%)	0.009
NHC (9% emulsifier)	0.012
NHC	0.012
NHC (anhydrous)	0.012
HS	0.005

Table 4.19. Saturation concentration of hydrocortisone in WHS (50% water), NHC (9% emulsifier), NHC, anhydrous NHC and HS

4.2.5. Summarized discussion of release experiments

From the results in this chapter the factors affecting drug release from semisolid preparations could be summarized as follows:

a. Base type:

The emulsion type of the base whether hydrophilic (o/w) or lipophilic (w/o) was found to directly influence drug release. For all the investigated systems it was proven that the liberation profiles of the o/w bases were significantly greater than those of the w/o systems. This was attributed to the significant greater solubility of the hydrocortisone in these bases and its fast release from the external aqueous phase.

The o/w systems investigated in this study were WHS and NHC 1%, both showed a significant high liberation profile without being significantly different. All dilution ratios of WHS 1% with WHS and NHC showed accordingly high liberation rates being lower

than those of the model cream WHS 1% which was referred to the difference in concentration gradient between suspended and dissolved parts of the drug upon dilution.

The dilution of WHS with WWS and HS in the ratio 1:1 resulted also in hydrophilic systems as was ensured by colouring and conductivity tests resulting in significantly higher liberation rates than the further dilutions which were proven to be converted into the w/o system.

All other dilutions of WHS 1% being lipophilic in character showed very low drug release.

b. Saturation concentration of the drug in the base:

All the tested vehicles are suspension type therefore the degree of solubility of the drug in the base is considered to play a great role in drug release. All the bases in this study which showed high liberation rates were associated with relatively high saturation concentrations of hydrocortisone.

However, an exception was observed in the case of the combination WHS/WWS 1:1 as the low saturation concentration of the drug in this base did not match with its relatively high liberation rate. It is believed that the effect of the emulsion type, being an o/w system thus releasing the drug freely from the outer aqueous phase, exceeds the effect of drug solubility.

c. Type of emulsifier:

The systems WHS/HS 1:1 and WHS/WS 1:1 in comparison to WHS/white petrolatum 1:1 showed the effect of adding a hydrophilic and a lipophilic emulsifier to white petrolatum respectively. The hydrophilic emulsifier retained the hydrophilic character of the base at a given water content and in addition it increased the amount of dissolved drug. Both factors together resulted in a pronounced greater liberation profile than the formulation WHS/WS 1:1 in which hydrocortisone is emulsified by a lipophilic emulsifier.

The type of emulsifier being non-ionic or ionic plays also an important role in drug release. It was found that the non-ionic surfactant in NHC possesses a greater influence on drug release and solubility of hydrocortisone than does the anionic emulsifier in WHS. Besides, in contrast to WHS, it was noticed that for NHC being emulsified by a non-ionic emulsifier the solubility of hydrocortisone is not affected by the amount of water present in the base.

d. Water content in o/w systems:

Water content has a major influence on drug release but only when incorporated in o/w systems. It was observed that reducing water content resulted in all cases in a significant reduction in drug liberation.

In almost all cases the drug was proven to be dissolved to a great extent in the external aqueous phase thus reducing the amount of this phase was always accompanied by a decrease in the amount of hydrocortisone dissolved leading thereby to decreased liberation rates. This effect was obviously seen in the systems WHS (50%) and WHS/HS 1:1 (35%) which showed a remarkable reduction in release rates of hydrocortisone compared with the original preparation WHS (70%).

The only exception was observed in the case of NHC anhydrous which has exactly the same solubilizing capacity for hydrocortisone as NHC with 50% water content thus the increased viscosity is probably the reason of the reduced drug release in this case.

d. Viscosity of the base:

The viscosity of the base, which is believed to have a great influence on drug release is discussed separately in the next chapter.

The factors, which were found to have no influence on drug release could be summarized as follows:

a. Order of dilution:

Incorporating the drug in either of the two bases before dilution was proven to have no influence on drug release from the diluted formulation, indicating that the distribution of the drug in the final preparation is independent of its distribution in the base before dilution.

b. Water content in w/o systems:

The systems WWS 1%, WHS/WWS 1:2 & 1:3, WHS/HS 1:1 & 1:2, all dilution ratios of WHS/WS and WHS/white petrolatum 1:1 are all w/o systems with different amounts of water, however they all showed significant low liberation rates which all lay in the same range. In conclusion, once water is fixed inside the base, the drug must diffuse through the external more viscous lipophilic phase, which is believed to be the rate limiting step, therefore any variations in its amount have no influence on drug release.

4.3. Rheological examination of the cream bases

A very important factor known to directly influence the drug release from semisolid preparations is the rheological behaviour of the bases (Korbar et al., 1982; Hsu et al., 1994; Fang et al., 1996; Fang et al., 1999).

Oscillatory measurement seems to be a suitable method to characterize the systems for release experiments, because it gives information about the viscous as well as the elastic properties of the vehicle, thus one could estimate their effect on drug release. Furthermore, it could help detecting and explaining structural changes following cream base dilutions. Moreover, oscillatory measurement is performed in the rheological ground state (viscoelastic range) of the material in which the structure of the base is still intact. The release of active ingredients from vehicles are intuitively presumed to have a closer correlation with the static state than the structurally destroyed state of the vehicle.

The viscoelastic range varies from one base to the other, therefore it was determined for each base separately by continuously increasing the shear stress at a constant frequency. At the critical stress point the linear viscoelastic range ends and the parameters e.g. phase angle, complex viscosity and moduli change their values drastically.

WHS 1% is taken as an example to illustrate the viscoelastic range (Fig. 4.19.). The falling of the straight line of the elastic modulus indicates the end of the range i.e. the vehicle begins to flow.

All systems were investigated in the linear viscoelastic range at a shear stress of 35 Pa except for HS 1% and all WHS/HS combinations, which were tested at 150 Pa, and NHC anhydrous, which was tested at 100 Pa because at lower shear stress values the measured parameters fluctuated strongly. The experiment is described in detail in 3.2.11.

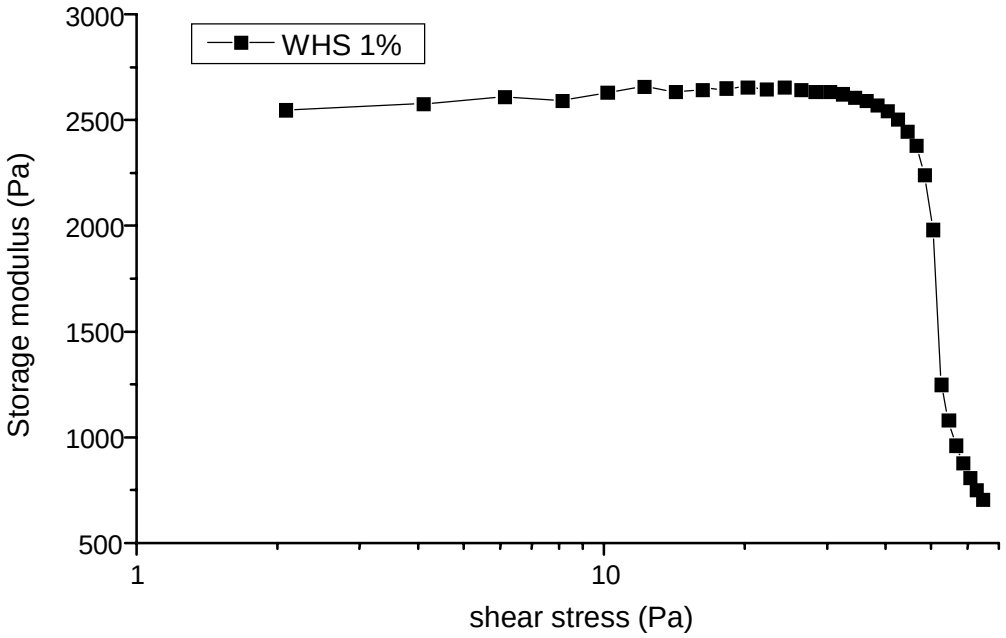


Fig. 4.19. Determination of viscoelasic range of WHS 1%

4.3.1. Oscillatory measurements of the different cream bases

The vehicles WHS, WWS, HS, WS and NHC with 1% hydrocortisone were investigated. The parameters determined were complex viscosity, which is the viscosity of both the viscous and the elastic parts of the base, phase angle, storage modulus and loss modulus.

The phase angle δ is an indicator for the overall viscoelastic nature of the sample ranging from 0° for an ideal elastic solid to 90° for a completely viscous liquid. As seen in Fig.4.20. the phase angle for all investigated preparations was lower than 45° which indicates that the elastic behaviour of the bases exceeds the viscous one.

This finding can also be detected by regarding both moduli (Fig. 4.21.). The storage modulus G´ describes the elastic behaviour of the vehicle whereas the loss modulus G´´ characterizes the viscous one. For all samples G´ was greater than G´´.

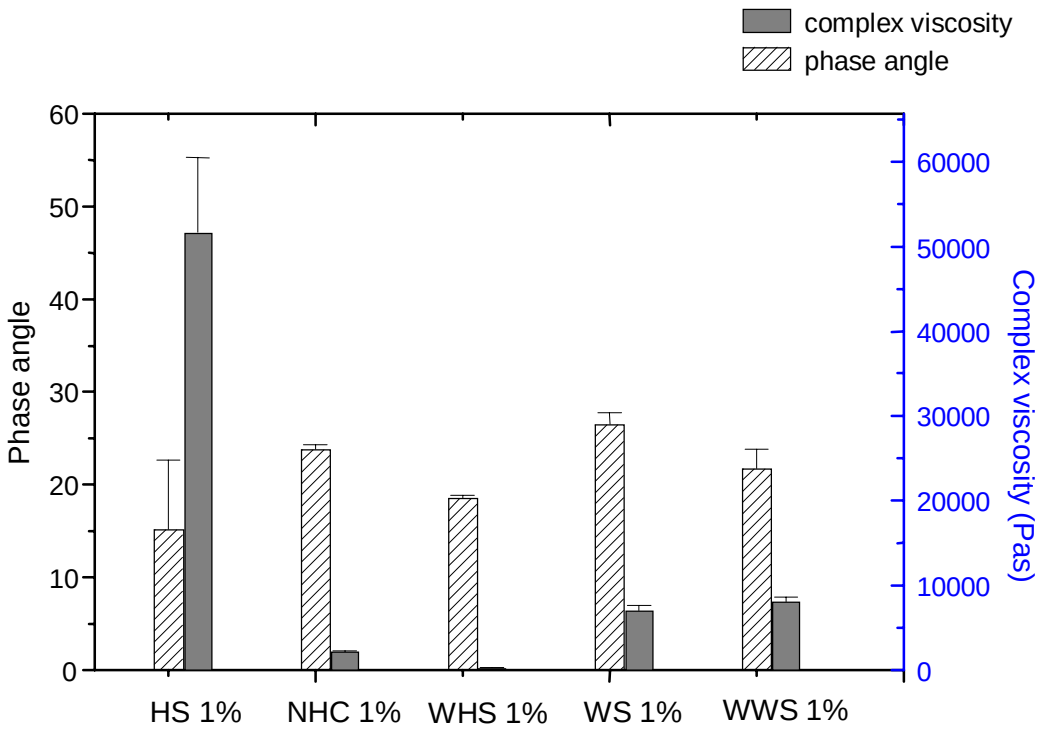


Fig. 4.20. Phase angle and complex viscosity of 1% hydrocortisone concentrated HS, NHC, WHS, WS and WWS

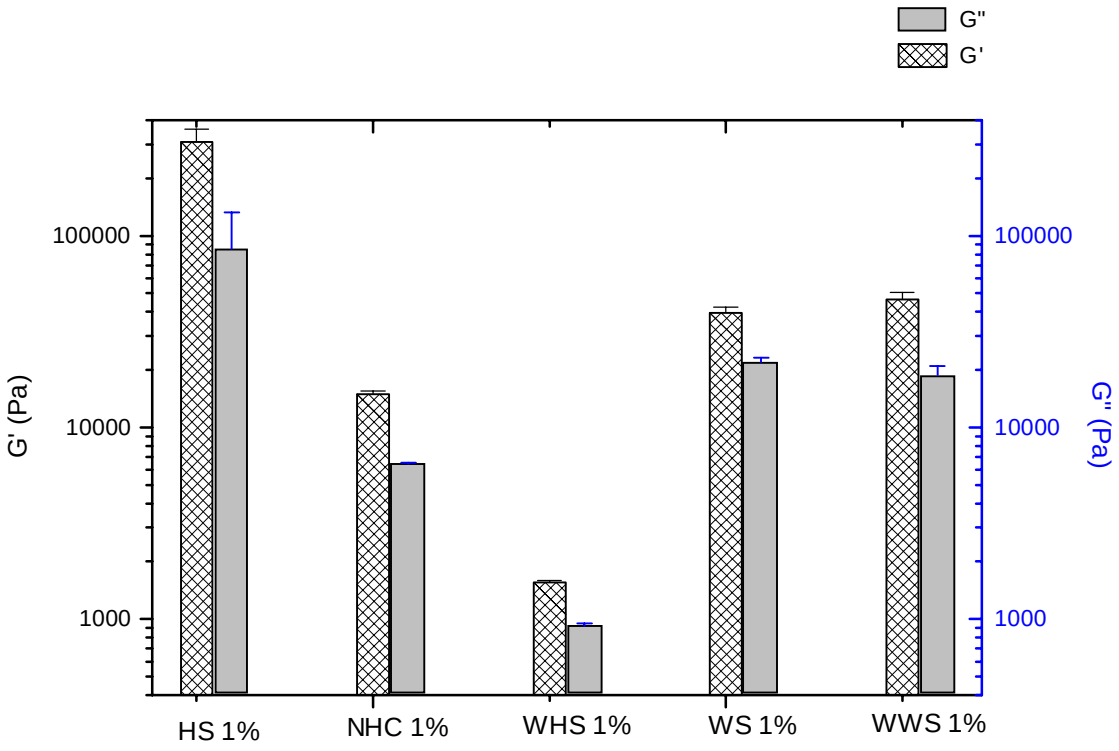


Fig. 4.21. Storage modulus and loss modulus of 1% hydrocortisone concentrated HS, NHC, WHS, WS and WWS

Regarding the complex viscosity of the bases (Fig. 4.20) and comparing it with the release experiments it is found that HS possesses the greatest viscosity among all bases. Both lipophilic bases (WS and WWS) exhibited a remarkably lower viscosity than that of HS, about 5-6 times lower, without being significantly different.

This finding agrees only partly with the release experiments as the release rate of hydrocortisone from the lipophilic bases was indeed almost identical, however it was lower than that of HS. In conclusion, the effect of base type and drug solubility in this case exceeds the effect of viscosity.

The very low viscosities exhibited by the hydrous hydrophilic vehicles match with their greatest releasing profiles, nevertheless, the viscosity of NHC being significantly greater than that of WHS does not agree with the identical release rate of both bases. The greater viscosity of NHC is probably compensated by the drug release-promoting effect of the non-ionic emulsifier in NHC resulting in identical drug liberation to that of WHS.

4.3.2. Oscillatory measurements of the diluted cream bases

4.3.2.1. Oscillatory measurements of WHS 1% diluted with WHS

Fig. 4.22. shows that using the same base for dilution results in neither a change in viscosity nor an alteration in the phase angle. This result was expected, however the experiment was carried out in order to investigate whether a double stress of the base would lead to any variations in the rheological behaviour of the bases. This double stress arises from using the Unguator twice, the first time for dispersing the drug in the base and secondly for diluting the base.

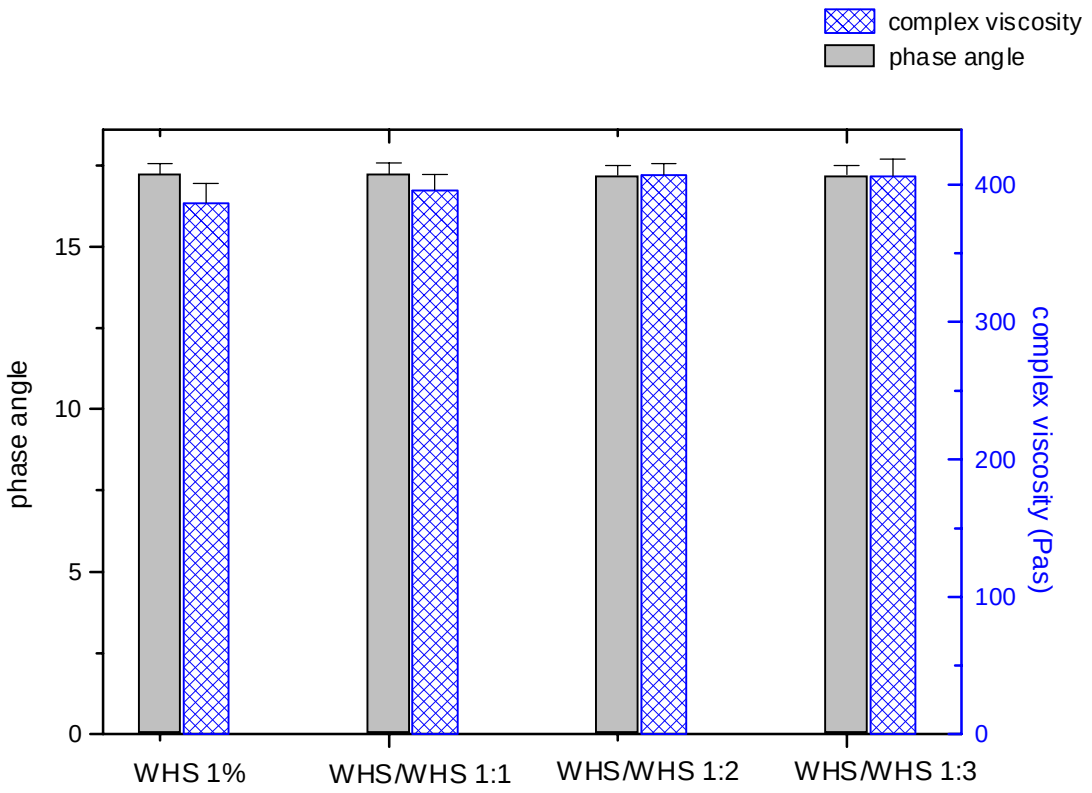


Fig. 4.22. Phase angle and complex viscosity of WHS 1% diluted 1:1, 1:2 and 1:3 with WHS

4.3.2.2. Oscillatory measurements of WHS 1% diluted with HS

Fig. 4.23. reveals that the phase angle of WHS/HS 1:1 having a value of about 22° is quite close to that of WHS 1% (18°); however, a significant increase in δ to about 30° is observed for the combinations 1:2 and 1:3. As mentioned previously a phase conversion takes place for these two formulations (4.2.2.1.4.). In the resulting w/o-type vehicles the structure of the systems completely changes and water is presumably dispersed as droplets in the vehicle.

It is known that there are several factors such as volume fraction of the disperse phase, droplet size, droplet size distribution, internal phase and continuous phase rheology, the interfacial rheology of the emulsifier film and the concentration and nature of the emulsifier that may be associated with the rheological properties of the

cream (Tadros, 1994). Therefore the structural change which occurred upon the 1:2 and 1:3 dilution would surely affect the elastic and viscous behaviour of the bases resulting in this significant change in phase angle.

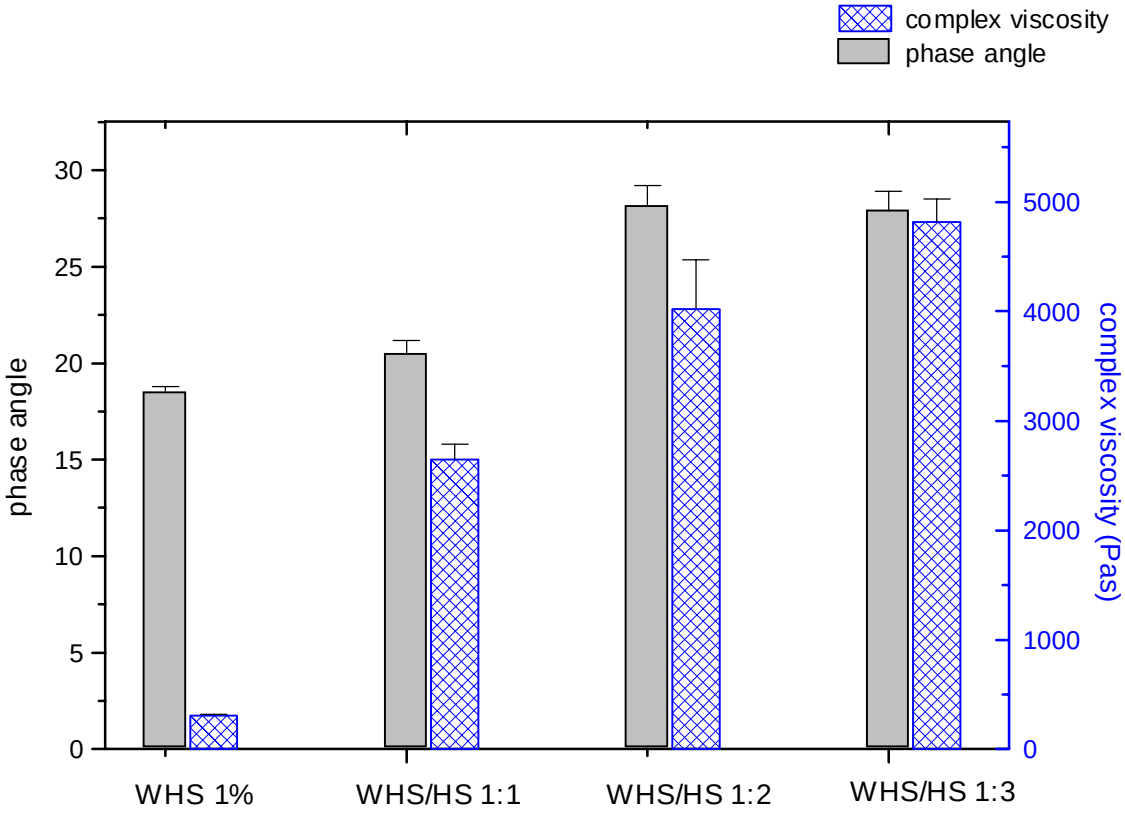


Fig. 4.23. Phase angle and complex viscosity of WHS 1% diluted 1:1, 1:2 and 1:3 with HS

This increase in δ would indicate an increase in the viscous behaviour of the formulations, however from Table 4.20. it is obvious that dilution resulted in an enormous increase in the values of the loss modulus G´´ and the storage modulus G´ as well. The greater δ indicates only that the increase of G´´ is greater than that of G´. For the 1:1 formulation, which did not suffer from great structural changes, the balance between loss and storage modulus was not greatly affected, however the values of both moduli increased, too. The increased elasticity of the creams is associated with increased consistency and viscosity of the vehicles (Tamburic et al,

1996). The great increase in viscosity observed for all preparations correlated very well with the decreased drug liberation from these formulations.

Base	Storage modulus G´ (Pa)	Loss modulus G´´ (Pa)
WHS 1%	1873.5 ± 32.1	629.1 ± 3.3
WHS/HS 1:1	15617.7 ± 786.7	5840.3 ± 321.4
WHS/HS 1:2	22329.1 ± 2613.1	11913.0 ± 1093.3
WHS/HS 1:3	26785.0 ± 1204.9	141460.8 ± 665.73

Table 4.20. Storage modulus and loss modulus of WHS 1% diluted 1:1, 1:2 and 1:3 with HS

4.3.2.3. Oscillatory measurements of WHS 1% diluted with NHC

No change of the phase angle (about 18°) occurred when WHS 1% was diluted with NHC (Fig. 4.24.), which means that the ratio between viscous and elastic properties remained constant upon dilution. This finding matches with the structural similarity between both bases (Niedner and Ziegenmeyer, 1992). However, the constant phase angle does not mean constant elastic and viscous properties as it is noticed from Table 4.21 that dilution resulted in increased viscous and elastic behaviour. The increased elasticity of the diluted vehicles was accompanied by a slight gradual increase in viscosity.

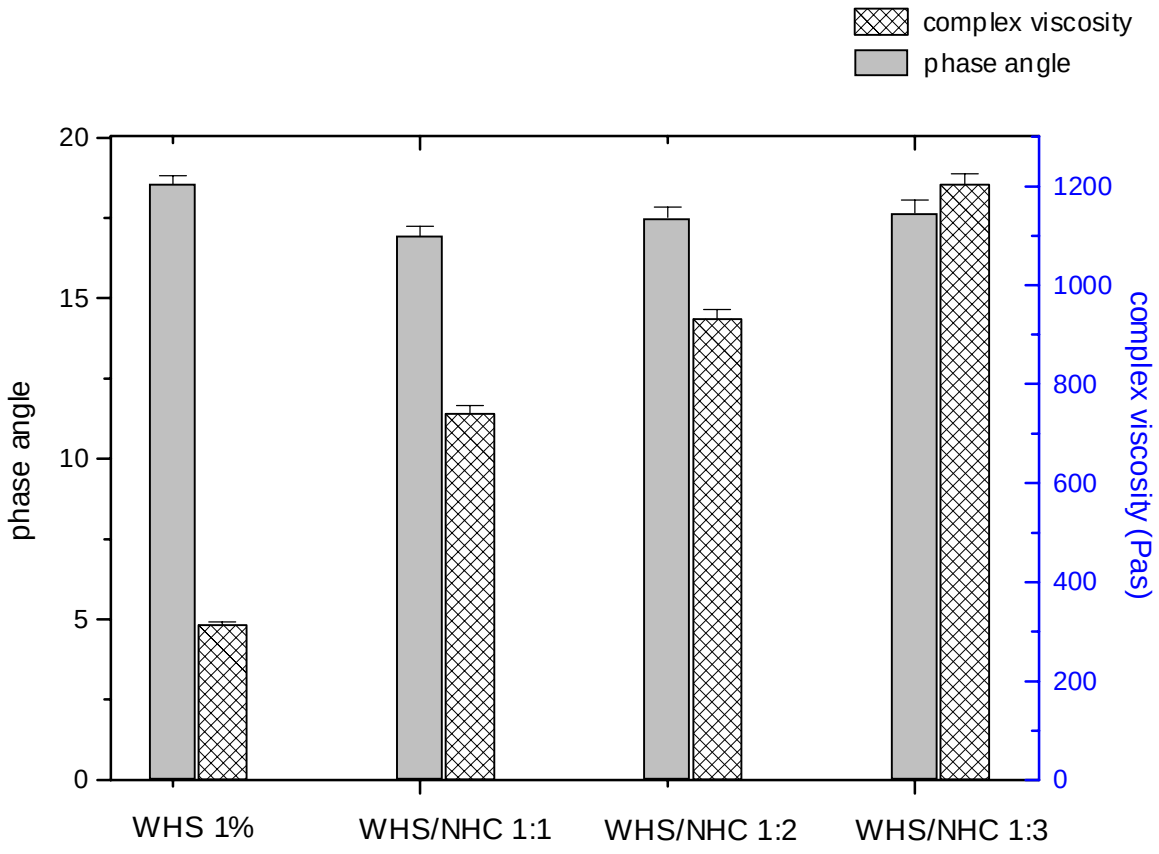


Fig. 4.24. Phase angle and complex viscosity of WHS 1% diluted 1:1, 1:2 and 1:3 with NHC

Base	Storage modulus G´ (Pa)	Loss modulus G´´ (Pa)
WHS 1%	1873.5 ± 32.1	629.1 ± 3.3
WHS/NHC 1:1	4456.8 ± 101.8	1357.13 ± 26.0
WHS/NHC 1:2	5590.3 ± 116.4	1764.9 ± 25.7
WHS/NHC 1:3	7212.1 ± 131.3	2297.9 ± 41.9

Table 4.21. Storage modulus and loss modulus of WHS 1% diluted 1:1, 1:2 and 1:3 with NHC

4.3.2.4. Oscillatory measurements of WHS 1% diluted with WWS

From Fig. 4.25. it is obvious that the combination WHS/WWS 1:1 revealed a phase angle almost identical to that of WHS 1% while a great increase in δ to about 30° is noticed for the dilution ratios 1:2 and 1:3. This rheological behaviour is quite similar to that observed for the diluted preparations of WHS with HS. Again here the phase conversion which was proven for the formulations 1:2 and 1:3 (4.2.2.1.2.) resulted in a disturbance in the balance between viscous and elastic properties leading to the significant change in phase angle. Despite of the increase in phase angle which means increased viscous properties, it is obvious from Table 4.22 that the elasticity of the bases greatly increased as well which would explain the immense increase in viscosity of these preparations. This great increase in viscosity correlates with the significant drop in drug liberation from these formulations.

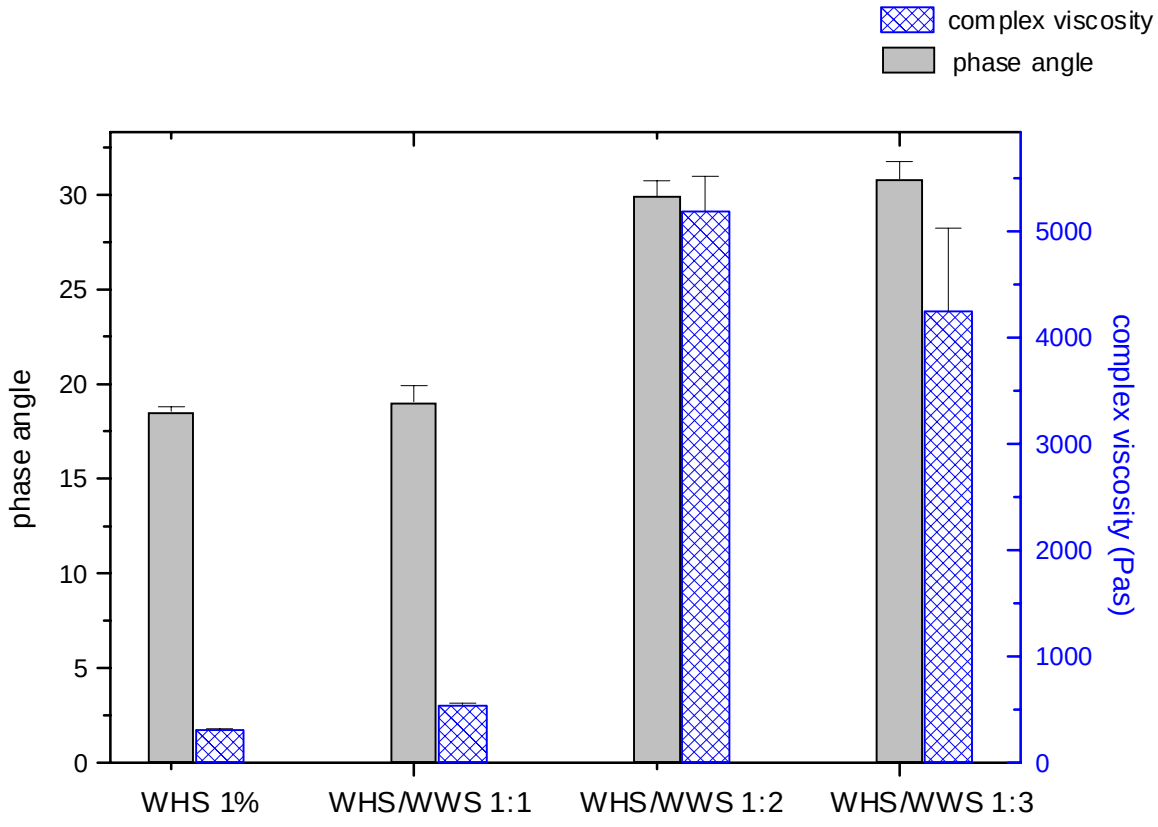


Fig. 4.25. Phase angle and complex viscosity of WHS 1% diluted 1:1, 1:2 and 1:3 with WWS

Base	Storage modulus G´ (Pa)	Loss modulus G´´ (Pa)
WHS 1%	1873.5 ± 32.1	629.1 ± 3.3
WHS/WWS 1:1	3246.7 ± 87.4	1122.9 ± 45.1
WHS/WWS 1:2	28278.1 ± 1901.9	16234.8 ± 890.4
WHS/WWS 1:3	22968.7 ± 4314.8	13661.4 ± 2330.1

Table 4.22. Storage modulus and loss modulus of WHS 1% diluted 1:1, 1:2 and 1:3 with WWS

On the other hand, the formulation WHS/WWS 1:1 showed only a slight increase in viscosity, much lower than that observed for WHS/HS 1:1. This would explain the greater hydrocortisone liberation from WHS/WWS 1:1 than from WHS/HS 1:1, despite of the greater solubility of hydrocortisone in the latter.

4.3.2.5. Oscillatory measurements of WHS 1% diluted with WS

A significant increase in phase angle to about 30° was observed for all three dilution ratios of WHS with WS (Fig. 4.26.) which is again an indication for the structural change of these formulations to the w/o system.

Again the immense increase in elasticity of the diluted preparations (Table 4.23.) resulted in a great rise in viscosity which correlates with the drastically reduced drug liberation of hydrocortisone observed for all WHS/WS combinations.

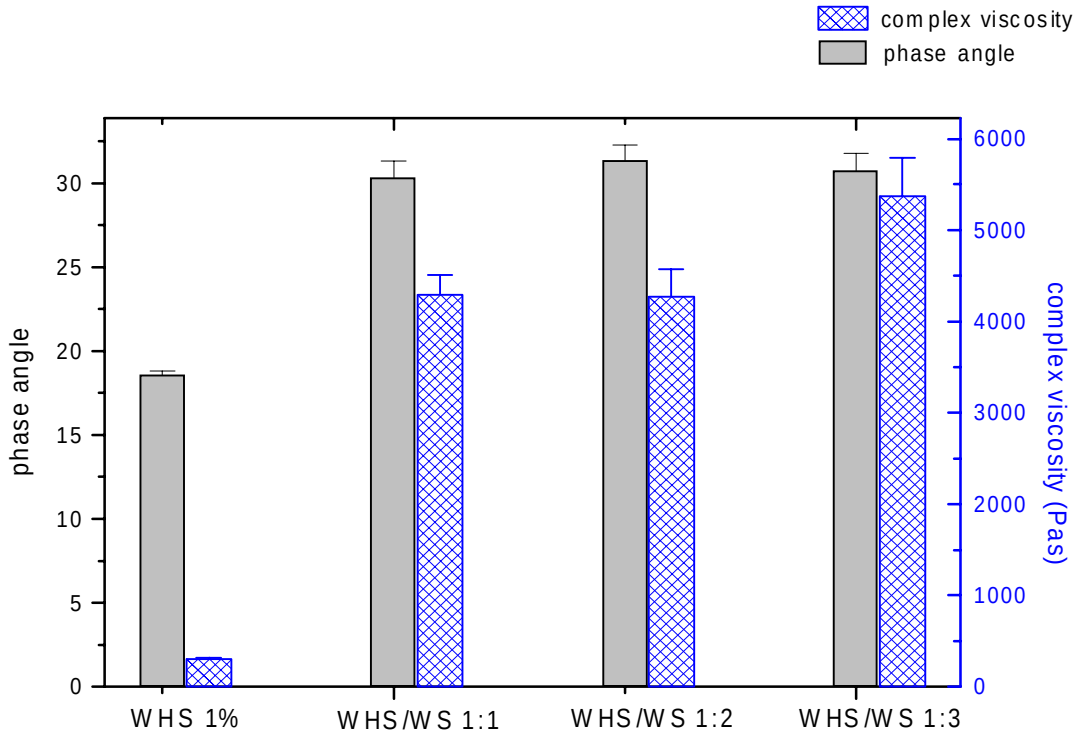


Fig. 4.26. Phase angle and complex viscosity of WHS 1% diluted 1:1, 1:2 and 1:3 with WS

Base	Storage modulus G´ (Pa)	Loss modulus G´´ (Pa)
WHS 1%	1873.5 ± 32.1	629.1 ± 3.3
WHS/WS 1:1	23324.2 ± 1293.4	13629.5 ± 553.4
WHS/WS 1:2	22955.5 ± 1715.6	13988.8 ± 858.7
WHS/WS 1:3	29071.2 ± 2410.7	17251.6 ± 1117.7

Table 4.23. Storage modulus and loss modulus of WHS 1% diluted 1:1, 1:2 and 1:3 with WS

4.3.3. Effect of water content on the rheological properties of a hydrophilic cream base

For this purpose the systems HS (50%), HS (30%) and HS (15%) with 1% hydrocortisone were investigated and compared with the model cream WHS (70%). As shown in Table 4.24. decreasing the amount of water to 50% resulted in a balanced increase in G´and G´´ i.e. insignificant change in the phase angle, which was accompanied by an almost 4 times increase in viscosity (Fig. 4.27.). This finding was also observed by other authors (Taleb and Erõs, 1996) who reported that in different o/w type creams the rheological characteristics increased exponentially by decreasing water content. The increase of viscosity could be regarded as a further explanation for the significant reduction in drug liberation, additional to the reduced hydrocortisone solubility in the base.

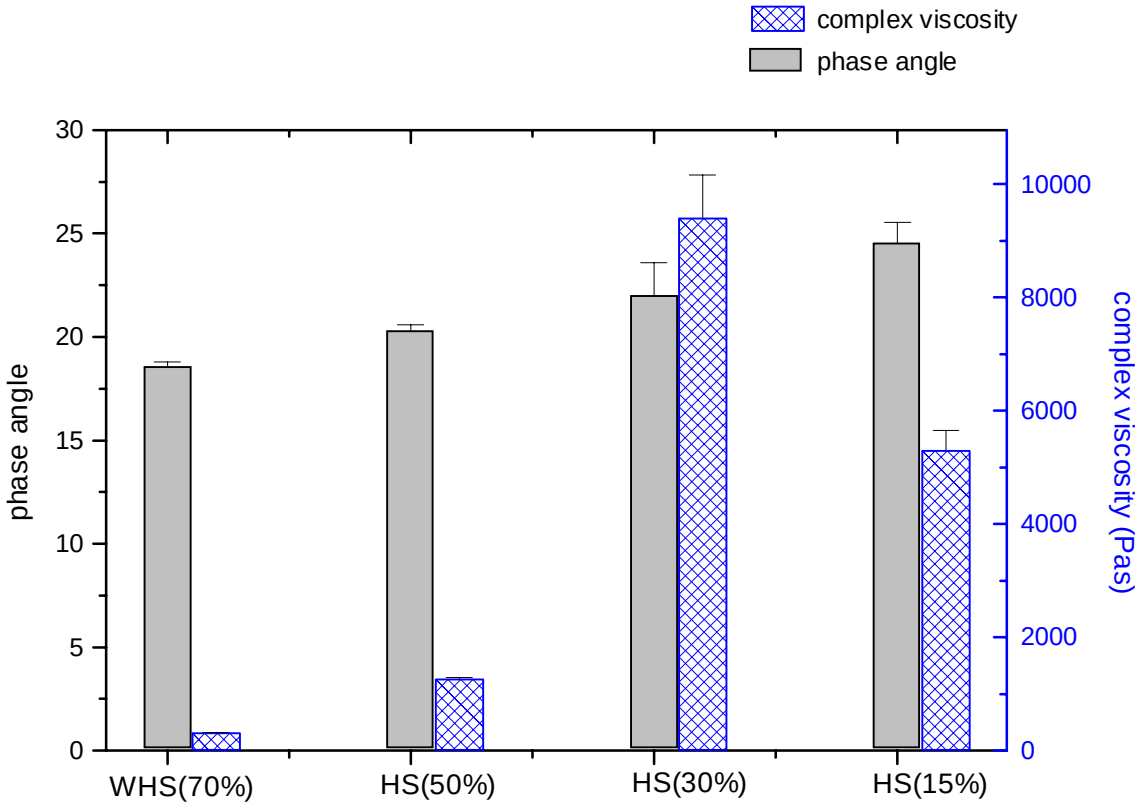


Fig. 4.27. Phase angle and complex viscosity of WHS (70%), HS (50%), HS (30%) and HS (15%)

Base	Storage modulus G´ (Pa)	Loss modulus G´´ (Pa)
WHS (70%)	1873.5 ± 32.1	629.1 ± 3.3
HS (50%)	7469 ± 137.3	2760.9 ± 35.5
HS (30%)	54776.8 ± 4387.2	22171.8 ± 2444.3
HS (15%)	30295.5 ± 2166.7	13820.1 ± 836.3

Table 4.24. Storage modulus and loss modulus of WHS (70%), HS (50%), HS (30%) and HS (15%)

The viscosity of the system HS 15% being around 5000 Pas lies in the same range as the viscosity of the systems WHS/HS 1:2 and WHS/HS 1:3 which contain 23.3% and 17.5% water, respectively. For these vehicles being all w/o systems, water content does not have a significant influence on viscosity.

Surprisingly, HS 30%, which was proven to be also a w/o system, showed a remarkably higher viscosity (about 9400 Pas). This finding may be attributed to the high instability of this system being on the border between the o/w and the w/o character as shown before (4.4.3.). This was also noticed optically, as this base was not easily prepared and it sometimes broke down during preparation.

However, it is to be noticed that the phase angle of the formulations HS (30%) and HS (15%) increased only slightly (Fig. 4.27.) and not as much as observed for the systems WHS/HS 1:2 and WHS/HS 1:3 despite of being probably very similar in structural arrangement. This observation may be referred to the difference in the method of preparation. The diluted systems were prepared by mixing HS with the already prepared WHS on cold, while HS (30%) and HS (15%) were prepared by directly incorporating the water phase to HS on hot which may lead to different characters such as droplet size and droplet distribution.

The effect of water content on the rheological properties was also investigated for NHC. From Fig. 4.28. it is obvious that NHC anhydrous possesses an enormously greater viscosity than NHC proving thereby that the increase in viscosity is indeed the reason why NHC anhydrous reveals a lower liberation rate for hydrocortisone than NHC despite of both having the same solubilizing capacity for hydrocortisone.

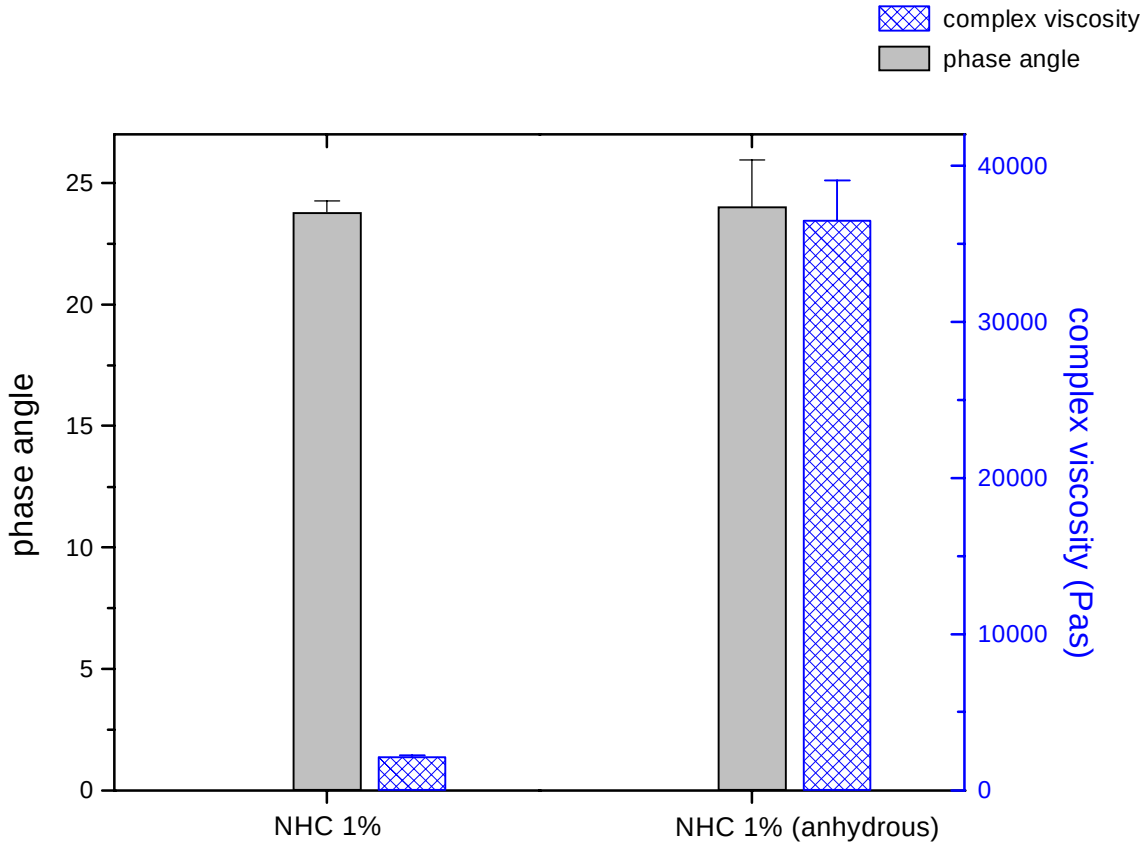


Fig. 4.28. Phase angle and complex viscosity of NHC 1% and NHC 1% (anhydrous)

4.3.4. Effect of emulsifier concentration on the rheological properties of a hydrophilic cream base

In order to investigate the effect of the emulsifier concentration on the rheological properties of the base, NHC, which normally contains 15% of non-ionic emulsifier, was compared with NHC containing only 9% emulsifier.

It is to be noted that reducing the amount of surfactant resulted in a significant decrease in the elasticity of the base by almost the factor four (Table 4.25.). This was associated with a similar decrease in viscosity (Fig. 4.29), which would mean

increased diffusivity of the drug within the vehicle. From this result one would expect a greater drug release for NHC (9%) over NHC (15%), however it must be taken in consideration that the emusifier contributes in promoting drug release, thus an increase in its concentration would also increase drug release. Therefore it is suggested that these two opposing effects compensated each other and led finally to an almost identical liberation profile for both bases.

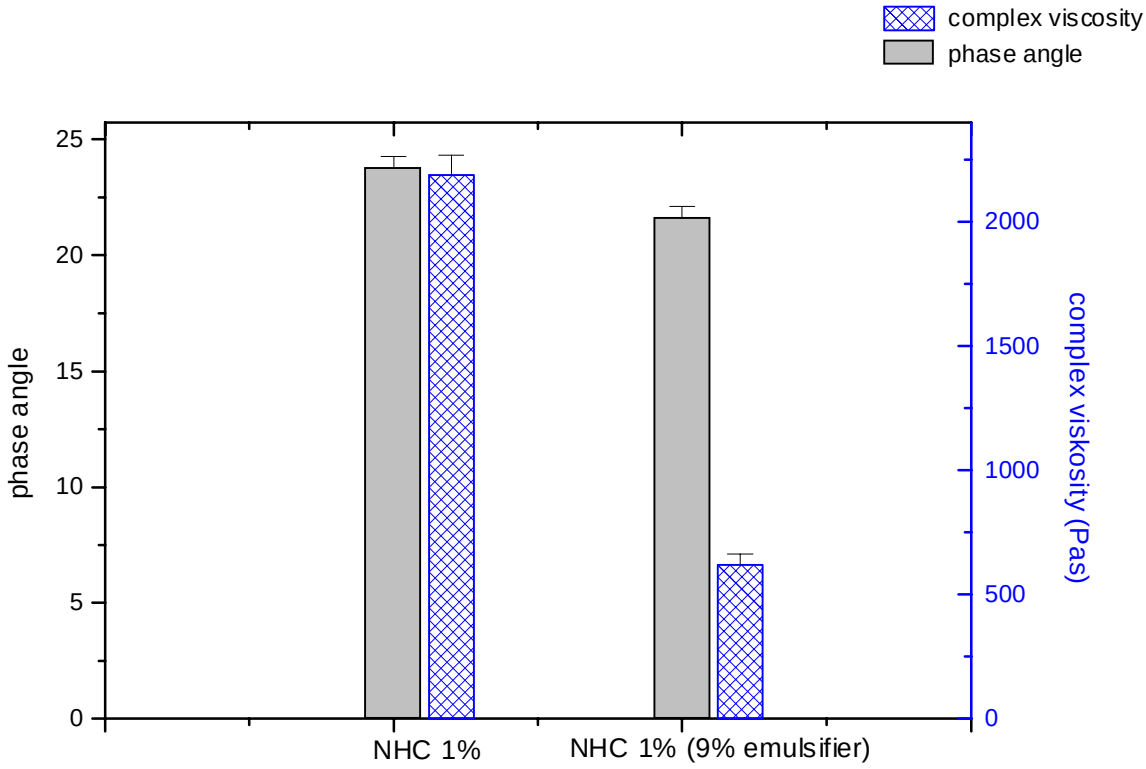


Fig. 4.29. Phase angle and complex viscosity of NHC 1% and NHC 1% (9% emulsifier)

Base	Storage modulus G´ (Pa)	Loss modulus G´´ (Pa)
NHC (1%)	15059.4 ± 396.2	6448.1 ± 126.3
NHC 1% (9% emulsifier)	3634.6 ± 226.2	1440.4 ± 111.2

Table 4.25. Storage modulus and loss modulus of NHC 1% and NHC 1% (9% emulsifier)

4.3.5. Summarized discussion of the rheological investigation and its correlation with the release experiment

From the previous investigations the base type with regard to hydrophilicity, lipophilicity and composition, as well as drug solubility in the base were found to have a great influence on drug release. In addition to all these factors the rheological behaviour of the vehicles was proven to have also a direct influence on drug liberation. However, sometimes the effect of one of the factors exceeds the effect of the other factors leading to some contradictions.

Regarding the complex viscosity of the undiluted bases it was found that the effect of base type on drug release dominates over the effect of viscosity. The lipophilic bases (WWS and WS), in spite of having much lower viscosities than HS, possess lower release rates for hydrocortisone.

Moreover, the viscosity of NHC being greater than that of WHS does not agree with their identical drug release especially in context that they have similar microstructure and identical dissolving capacity for hydrocortisone i.e. probably the release promoting effect of the emulsifier in NHC is compensated by its increased viscosity. The greater viscosity of NHC over WHS may be attributed to the reduced amount of water in NHC. NHC, with its 50% water content, has a comparable viscosity with that of WHS which contains 50% aqueous phase.

The dilution of WHS 1% with WS, HS and WWS with the exception of the combinations WHS/HS 1:1 and WHS/WWS 1:1 showed a phase conversion to the w/o system as proven in the previous chapter. This great structural change was expressed in an inbalanced increase in both, the viscous and elastic properties of the vehicles leading to a significant increase in the phase angle to about 30°. This was accompanied by a great rise in viscosity which correlates with the enormous drop in drug release from these formulations.

The formulations WHS/HS 1:1 and WHS/WWS 1:1 which retained their hydrophilic character with no great structural changes, revealed an insignificant change in the phase angle. This was a result of a balanced increase in the values of both moduli. The increased elasticity of the vehicles was accompanied by an increase in viscosity, the extent of which differed from one base to the other. The viscosity of the formulation WHS/HS 1:1 was much greater than that of WHS/WWS 1:1 which may

be referred to the difference in water content. The relatively low viscosity observed for WHS/WWS 1:1 was accompanied by a relatively high liberation rate whereas the high viscosity of WHS/HS 1:1 was associated with a remarkably low liberation profile.
Diluting WHS with NHC was accompanied by a gradual increase in viscosity, which was considered to be the reason for the lower liberation profiles of WHS/NHC combinations compared to the corresponding WHS/WHS combinations in spite of possessing the same microstructure and saturation concentration of hydrocortisone. In this case the increased viscosity seems to be not compensated by the effect of the emulsifier (as discussed in the case of NHC) which may be attributed to its dilution (its partial substitution by the anionic emulsifier of WHS).
It is remarkable that the drug release from the dilution ratios of WHS 1% with NHC was greater than that of WHS/WWS 1:1 in spite of having higher viscosities. Considering the solubility of hydrocortisone in the different bases helps to explain this contradiction. The solubility of hydrocortisone in all WHS/NHC combinations is about 4 times greater than its solubility in WHS/WWS 1:1.
The great increase in viscosity upon reducing water content in the o/w bases may add a further explanation - additional to the reduced drug solubility – for the significant reduction in drug release. On contrast, investigations show that the amount of water in the w/o systems has no influence on viscosity; a finding which agrees with the very close liberation profiles of all tested w/o systems despite of having different water contents.
In addition, surfactant concentration was also found to have a direct influence on the rheological properties of the vehicle. Decreasing the concentration of the non ionic emulsifier in NHC led to a significant decrease in elasticity and viscosity of the vehicle.
Regarding the phase angle of the pure and diluted vehicles it seemed surprising that the phase angle of the pure bases (HS, WS, WWS) were all below 30°, however upon dilution the phase angle increased to above 30° for all bases showing a phase conversion. Considering the fact that the rheological properties of semisolid systems are greatly influenced by the ratio of their componenets (surfactants, hydrophilic and lipophilic phase) as well as many other factors as droplet size, droplet size distribution and nature and concentration of the emulsifier, one would suggest that dilution may result in systems having viscoelastic behaviour completely different from the pure bases.

4.4. Permeation experiments

The ability of a drug in a topical formulation to exert its effect is dependent on two consecutive physical events. The drug must first diffuse out of the vehicle to the skin surface, and then it must permeate this natural barrier to the site of action.
After the release of hydrocortisone from various cream bases, and the effect of dilution on drug release were investigated in detail in chapter 4.2. it seemed important to carry on the investigation and examine drug permeation through skin.
There are several possibilities in order to gain information about the permeation of hydrocortisone through the skin. The in vivo method widely used in the case of glucocorticoids is the skin blanching assay depending on their vasoconstrictive property. Alternative to the in vivo method is the in vitro permeation testing mainly through excised human skin or excised human stratum corneum.
The permeation experiments carried out in this chapter were done through excised human stratum corneum, which represents the main barrier of the skin, in order to examine the influence of vehicle composition and the effect of base dilution on hydrocortisone permeation. The permeation experiments were performed as described in 3.2.9.2.

4.4.1. Permeation of hydrocortisone from different cream bases

In order to examine the influence of base type on permeation of hydrocortisone through excised human stratum corneum and to reveal its correlation with the release experiments the same cream bases, WHS, WWS, NHC, WS and HS with 1% hydrocortisone were used, taking in consideration that these bases are examples of hydrophilic as well as lipophilic bases with and without water content. Thus enabling the investigation of most possible factors concerning vehicle composition that may exhibit an effect on drug permeation.

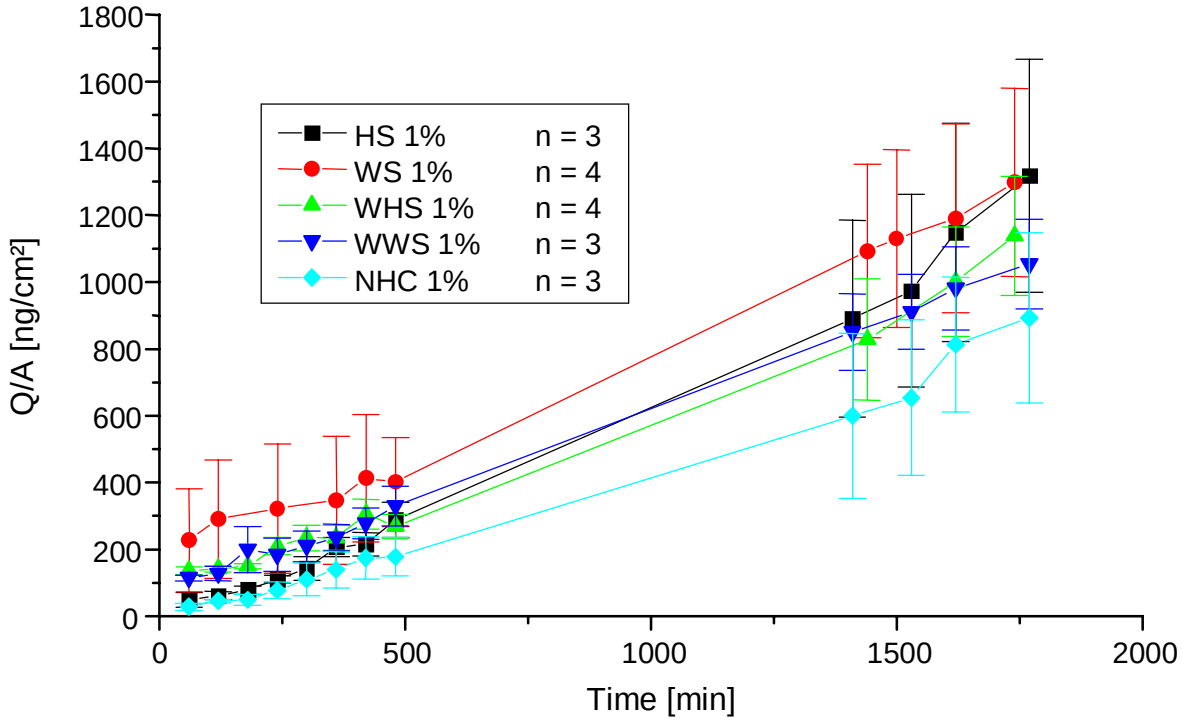


Fig. 4.30. Permeation of hydrocortisone from 1% WHS, WWS, NHC, HS and WS through excised human stratum corneum

From Fig. 4.30. it is evident that there is no significant difference between the various cream bases regarding the permeation of hydrocortisone through excised human stratum corneum. According to the equation 2.5. the drug flux [g/cm²*s] could be calculated from the linear part of the curves. In order to neglect the influence of the starting concentration c_0 as well as the saturation concentration c_s of the drug in the vehicle on permeation, the permeation coefficient P as well as the persol-coefficient Z were determined according to the equations 2.6. and 2.8., respectively.

The starting concentrations of hydrocortisone in the various vehicles are slightly different despite of being all 1% g/g concentrated. The reason for this contradiction is that, according to the equation 2.5., the concentration must be given in g/cm³. Therefore, differences in the densities of the various bases would lead to differences in the starting concentrations. The data are illustrated in Table 4.26.

Vehicle (1% hydrocortisone)	Flux J ($g/cm^2 * s) * 10^{-12}$	Permeation coef. P $(cm/s) * 10^{-9}$	Persol-coef Z $(cm/s) * 10^{-9}$
HS	12.35 ± 3.2	1.42 ± 0.37	273.72 ± 71.0
WS	11.38 ± 1.89	1.34 ± 0.22	1335.29 ± 222.4
WHS	9.72 ± 1.83	1.01 ± 0.19	84.6 ± 15.9
WWS	9.6 ± 0.75	1.06 ± 0.08	529.28 ± 41.6
NHC	7.34 ± 3.31	0.76 ± 0.35	63.82 ± 28.8

Table 4.26. Hydrocortisone flux, permeation coefficient and per-sol coefficient of 1% WHS, WWS, NHC, WS and HS

From the table it is obvious that WS 1% and HS 1% have greater fluxes and permeation coefficients than the other bases, but this observation is not significant as the standard deviations of the single curves overlap.

The great differences in the per-sol coefficient of the various bases varying from 1335,29 $* 10^{-9}$cm/s for WS 1% to 84.6 $* 10^{-9}$cm/s for WHS 1% though having similar permeation rates indicate the negligible role of the amount of drug dissolved in the bases on permeation through stratum corneum.

This finding allows the conclusion that the drug release experiments do not correlate with the permeation experiments through human stratum corneum, as the factors affecting drug release such as vehicle composition, drug solubility in the base and viscosity of the preparation do not play a role in drug permeation.

The lack of correlation between release and permeation experiments was also reported by other authors. Zatz et al. (1996) examined the release of betamethasone dipropionate from petrolatum-based ointments with two different formulations and

found that the ointment with the greater release was the less clinically effective of the two. Hsu et al. (1993) reported that the release of piroxicam from FAPG base decreased with addition of fatty acids to the base due to an increase in the lipophilicity and viscosity of the vehicle, whereas the addition of fatty acids increased the percutaneous absorption of piroxicam due to their enhancer effect. The influence of replacing different portions of the water content in WHS by ethanol on hydrocortisone acetate release and permeation through excised human stratum corneum was investigated (Alberg, 1998), no correlation was again found between permeation and release experiments.

In order to interpret these results it should be first considered, whether the drug is suspended or dissolved in the vehicle. In case of suspension bases the quantity Q of the drug diffused from the vehicle V through the skin barrier is calculated as follows (Lippold, 1981):

$$Q = \frac{D * F * VF * f_V * C_{sV}}{d} * t \qquad \text{(Eq. 4.1.)}$$

D = Diffusion coefficient of the drug in stratum corneum
F = Permeation area
VK = partition coefficient between barrier and vehicle
f_V = fraction of the drug in the vehicle available for permeation
c_{sV} = saturation concentration of the drug in the vehicle
d = thickness of stratum corneum
t = time

The above equation reveals a direct relationship between saturation concentration of the drug in the vehicle and the amount of drug permeating stratum corneum. Accordingly, one should expect a change in the vehicle i.e. change in the amount of the drug dissolved in the vehicle would be associated with an alteration in the substance transport. However, it should be taken in consideration that a change in the saturation concentration is accompanied by a reverse change in the partition coefficient.
The partition coefficient of a substance between an organic phase and an aqueous phase is described in equation 4.2.:

$$VK_{org/aq} = \frac{f_{org} * C_{org}}{f_{aq} * C_{aq}} = \frac{f_{org} * C_{sorg}}{f_{aq} * C_{saq}} \qquad \text{(Eq. 4.2.)}$$

f_{org} and f_{aq} are fractions of the substance available for diffusion (free, unbound) in the organic phase and in the aqueous phase, respectively.
Subsequently the partition coefficient of the drug between the stratum corneum barrier (B) and vehicle (V) is described as follows:

$$VK = \frac{f_B * C_{sB}}{f_V * C_{sV}} \qquad \text{(Eq. 4.3.)}$$

Resulting from equation 4.1. and 4.3. the transport of the drug through stratum corneum is illustrated in equation 4.4.:

$$Q = \frac{D * F * f_B * C_{sB} * f_V * C_{sV}}{d * f_V * C_{sV}} * t \qquad \text{(Eq. 4.4.)}$$

This is simplified to:

$$Q = \frac{D * F}{d} f_B * C_{sB} * t \qquad \text{(Eq. 4.5.)}$$

From the above equation it is evident that the factor affecting drug transport through stratum corneum is the saturation concentration of the drug in stratum corneum c_{sB} which is specific to the substance and not the solubility of the drug in the vehicle as long as there is enough free (dissolved) substance able to saturate the barrier. In conclusion, changing the vehicle does not influence drug permeation as proved in Fig. 4.30.

This finding was also observed by Schwarb et al. (1999), who reported that in contrast to the in vitro release testing for fluocinonide, the in vivo human skin blanching assay was found to be independent of the degree of saturation.

Furthermore, an early publication of Ostrenga et al. (1971) reported that polyacrylate gel containing 0.025% fluocinolone acetonide with varying proportions (5-25%) of propylene glycol/water mixtures -which act as solubilizing agent- revealed similar percutaneous absorption in spite of having different degrees of saturation due to variable propylene glycol content. This result is not surprising as it was found that up to 25% propylene glycol the drug is still suspended in the vehicle.

On the other hand, a significant influence of the vehicle on percutaneous absorption is observed when the drug is dissolved. The affinity of the drug to the vehicle in this case plays the major role on permeation. A great affinity between drug and vehicle is associated with a relatively low barrier/vehicle partition coefficient, accordingly a low permeation rate. In contrast, great permeation rates are expected in the case of low affinity between drug and vehicle (Lippold, 1981).

These facts altogether lead to the conclusion that as long as the drug is suspended in the base the degree of saturation, hence the type of vehicle does not influence permeation through excised stratum corneum.

Nevertheless, if the experiments were performed in vivo the effect of occlusion may play a role on drug permeation. The application of an ointment on the skin surface prevents evaporation of water and develops a state of increased hydration of the skin. Hydration results in increased drug penetration. Application of creams and lotions under normal conditions (non-occluded) allows free exchange of air and water, and the skin does not achieve the state of higher hydration. Thus different formulations may result in different amounts of drug permeation into the skin and may, thus, exhibit different intensities of activity (Shah et al., 1991). The authors reported an in vitro release of hydrocortisone in the order of lotion>cream>ointment, whereas the pharmacological response was in the reversed order.

All the above mentioned facts are true in the case of intact skin. If we look at damaged skin and mucosa the relations are completely different. Here the release is the rate limiting step for the drug uptake due to the absence or leakage of stratum corneum. When formulations are developed for damaged skin or mucosa, an

increase of the drug concentration, or raised drug solubility always increases the release rate and thereby the drug uptake (Lippold, 1984).

Furthermore, it must be taken in consideration that the vehicle composition is mostly altered when the formulation is applied on the skin, e.g. change in the water content due to evaporation or in the lipid composition due to possible interactions with the lipids of stratum corneum.

4.4.2. Permeation of hydrocortisone from diluted cream bases

In order to investigate the influence of dilution on hydrocortisone permeation through excised human stratum corneum WHS 1% was diluted in the ratio 1:1 with the same base, WHS, and the different chosen cream bases WWS, WS, NHC and HS.

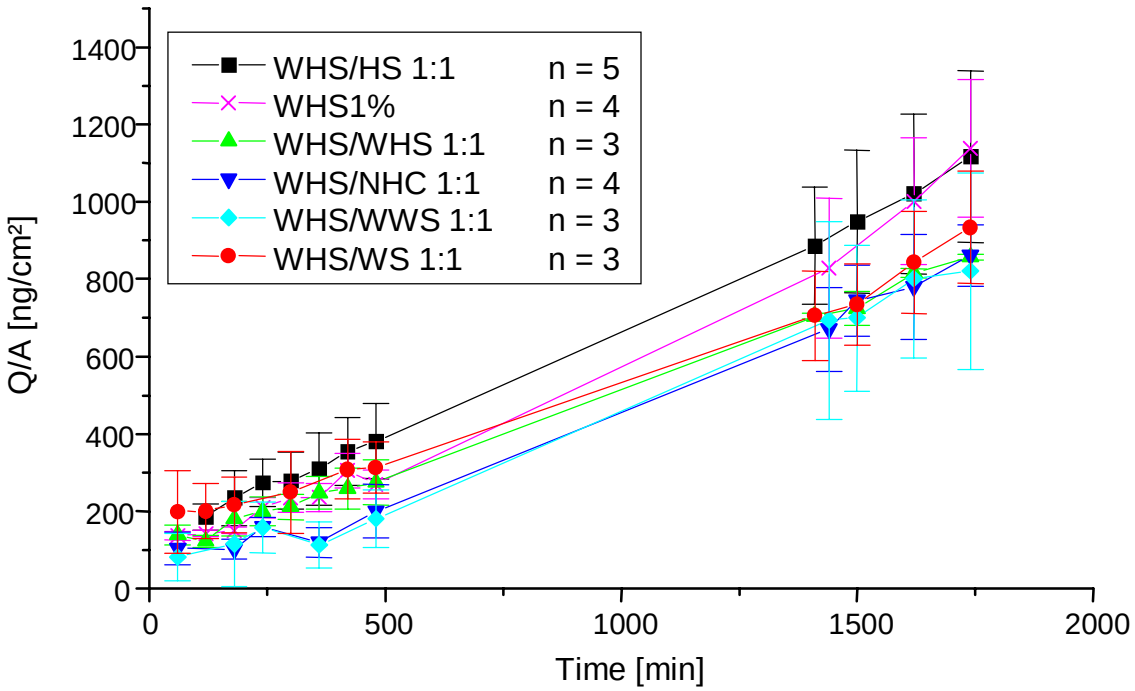


Fig. 4.31. Permeation of hydrocortisone from WHS 1% diluted 1:1 with WHS, WWS, NHC, WS and HS

From Fig. 4.31. it is clear that no remarkable differences can be detected between the permeation rates of all diluted formulations. This was expected as the permeation profiles of the different undiluted bases were similar as shown in Fig. 4.30. Furthermore, the permeation rates of the diluted as well as the undiluted (WHS 1%) preparations cannot be distinguished from each other, which reveals the negligible influence of dilution on permeation. The flux, permeation coefficient and the per-sol coefficient were again calculated and demonstrated in Table 4.27.

Vehicle	Flux J $(g/cm^2 * s) * 10^{-12}$	Permeation coef. P $(cm/s) * 10^{-9}$	Persol-coef Z $(cm/s) * 10^{-9}$
WHS 1%	9.72 ± 1.83	1.01 ± 0.19	84.6 ± 15.9
WHS/HS 1:1	9.28 ± 1.56	2.02 ± 0.35	154.63 ± 26.5
WHS/WS 1:1	7.8 ± 0.98	1.67 ± 0.21	433.33 ± 54.6
WHS/WHS 1:1	7.6 ± 0.57	1.58 ± 0.12	66.09 ± 4.9
WHS/WWS 1:1	7.68 ± 1.97	1.56 ± 0.40	236.31 ± 60.6
WHS/NHC 1:1	7.77 ± 1.32	1.66 ± 0.28	69.38 ± 11.78

Table 4.27. Hydrocortisone flux, permeation coefficient and per-sol coefficient of WHS 1% diluted in the ratio of 1:1 with the various cream bases.

Diluting WHS 1% 1:1 with all selected vehicles with exception of HS gave almost identical fluxes being significantly lower than that of WHS 1%. The relatively higher flux of the formulation WHS/HS 1:1 correlates well with the fact that HS 1% shows the highest flux among the undiluted bases (Table 4.26.). However, a similar effect was not observed with the dilution WHS/WS 1:1, although WS 1% has a higher flux when compared with the other bases. Taken together, these results suggest that all these slight differences in the fluxes of the diluted as well as the undiluted

formulations are not significant especially because the standard deviations of all single curves overlap.

In order to confirm this result, higher dilutions of WHS 1% were performed. WHS 1% was diluted with a lipophilic base, WWS, as well as with a hydrophilic base, NHC, in the ratio 1:3 and examined for permeation of hydrocortisone. The results are shown in Fig. 4.32 and Fig. 4.33, respectively.

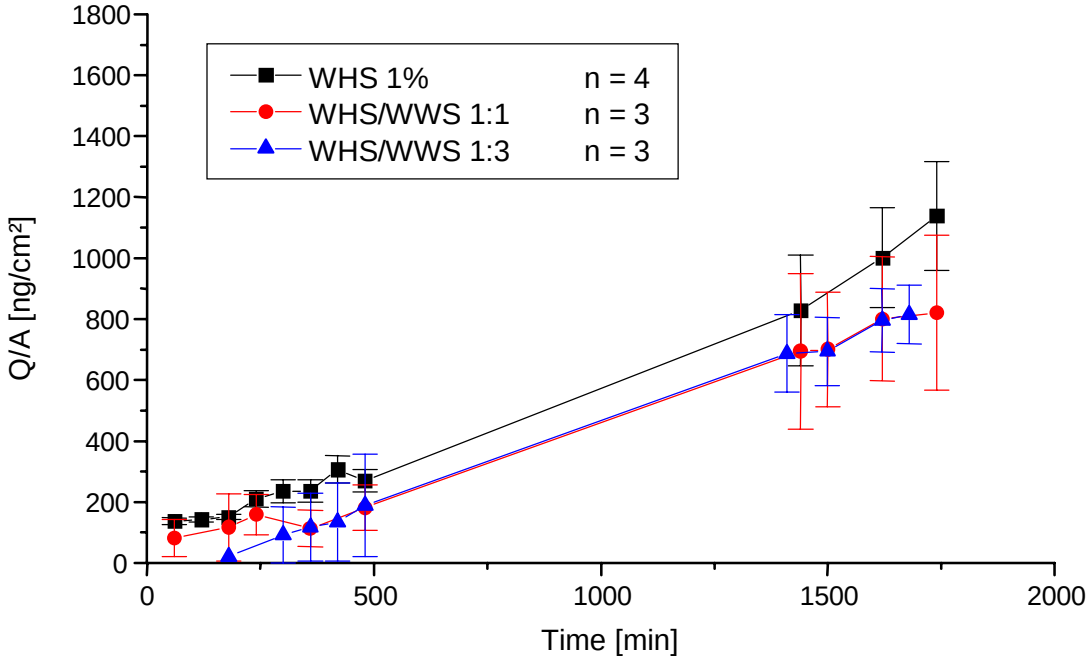


Fig. 4.32. Permeation of hydrocortisone from WHS 1% diluted 1:1 and 1:3 with WWS

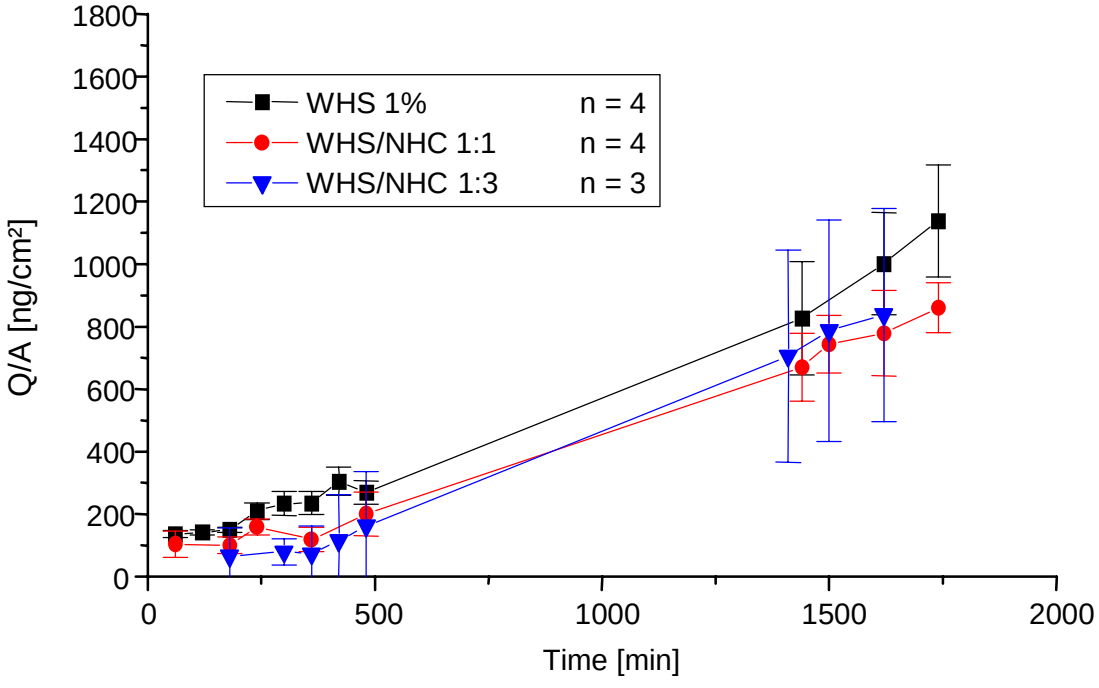


Fig. 4.33. Permeation of hydrocortisone from WHS 1% diluted 1:1 and 1:3 with NHC

The figures show that even the 1:3 dilution of WHS 1% with either of the two bases chosen does not lead to a reduced permeation of hydrocortisone. These results suggest that the permeation of hydrocortisone through excised human stratum corneum is independent of the drug concentration in the applied base in addition to the type of vehicle as mentioned previously. A similar observation was reported by Stoughton and Wullich (1989) who reported that higher glucocorticoid concentrations do not cause higher activity or bioavailability.

It must be considered that hydrocortisone in all diluted formulations is still suspended in the vehicle to a great excess (Table 4.2.) i.e. the dilution only reduced the amount of the drug suspended in the base, whereas the vehicle is still saturated with the drug. According to equation 4.5. the permeation rate of a drug through stratum corneum is directly influenced by its saturation concentration in the stratum corneum c_{sB} which is specific to the drug. As long as the same drug is used for permeation, the c_{sB} is retained unchanged, thus no change should be expected in the permeation even if the vehicle or the concentration of the drug in the vehicle are altered. According to these considerations the amount of drug suspended in the vehicle whether high or low does not play a role on drug permeation through stratum corneum (Lippold, 1981) i.e. in order to influence drug permeation very high dilutions (up to 1:100) should be performed to reduce the drug content in the vehicle below its saturation concentration. These dilutions were not carried out in this work as they are not done in practice.

These data are in agreement with those of Magnus et al. (1981) who reported that Betnovate® cream diluted with several cream bases showed good stability and the potency of the dilutions were equivalent to the undiluted cream. Similar dilutions of Betnovate ointment (Ryatt et al., 1983) with Unguentum Merck did not reduce the potency of the ointment up to a dilution of 1:32.

Moreover, the vasoconstrictive effect of fluocinolone acetonide in the commercially available Synalar® preparations (gel, cream and ointment) was investigated (Gao and Li Wan Po, 1994) (marketed in Germany under the brand name Jellin®). The (1 in 4) and (1 in 10) diluted products appeared to be of the same potency as the full strength cream. The authors suggested that even at the 1 in 10 dilution, sufficient corticosteroid is present to saturate the receptors of corticosteroids in the skin, so that an increase in concentration will not produce any enhancement in activity.

Generally, in an attempt to overcome the problems arising from skin impermeability, various approaches to reversibly remove barrier resistance have been investigated. Among these approaches is the use of permeation enhancers, which are known to allow drugs to permeate more readily to the viable tissue (Golden et al, 1986; Leopold et al, 1995; Gorukanti et al, 1999). Therefore, it was interesting to investigate the influence of diluting a vehicle containing an enhancer on hydrocortisone permeation through excised stratum corneum.

Among the great number of important enhancers reported in literature (Barry, 1987a), isopropyl myristate as an example of fatty acid esters was chosen for its pronounced enhancing effect (Gorukanti et al., 1999). Soventol® cream, which contains isopropyl myristate was found to significantly promote the permeation of hydrocortisone acetate through stratum corneum when compared with other commercial products (Alberg, 1998). It is to be noted that Soventol® cream contains also isopropyl alcohol which is known to possess also an enhancing effect. Therefore, Soventol cream placebo prepared with 1% hydrocortisone and its 1:2 dilution with WHS were tested for permeation of hydrocortisone in comparison to WHS 1%. The results are shown in Fig. 4.34 and Table 4.28.

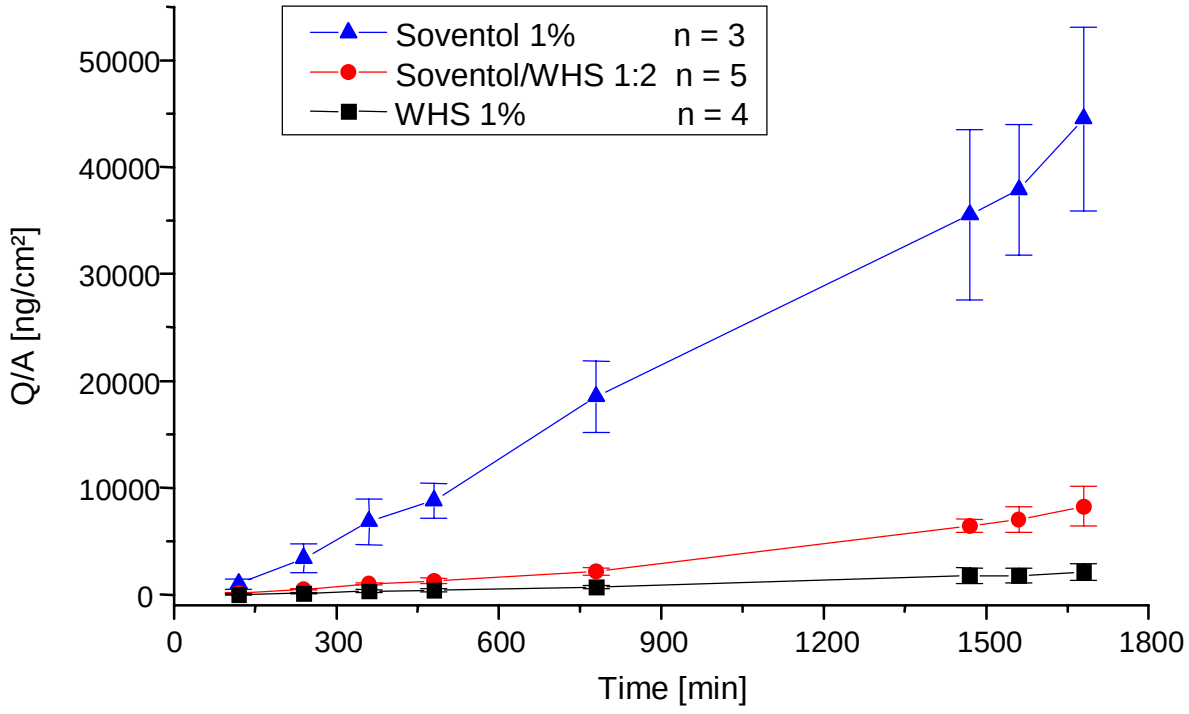


Fig. 4.34. Permeation of hydrocortisone from 1% Soventol cream, Soventol cream diluted with WHS 1:2 and 1% WHS

From Fig. 4.34. it is to be noted that hydrocortisone is permeating significantly better from Soventol cream than from WHS. As shown in Table 4.28. the permeation of hydrocortisone from Soventol cream is about 20 times greater than that of WHS proving thereby the influence of the permeation enhancer.

Vehicle	Flux J g/cm^2*s*10^{-10}	Perm.coef. P $cm/s*10^{-9}$
Soventol 1%	4.5 ± 0.8	43.06 ± 7.65
Soventol/WHS 1:2	0.85 ± 0.15	25.87 ± 4.61
WHS 1%	0.23 ± 0.07	2.39 ± 0.71

Table 4.28. Hydrocortisone flux and permeation coefficient of 1% Soventol cream, Soventol cream diluted with WHS 1:2 and 1% WHS

Isopropyl myristate being a long-chain saturated fatty acid ester was shown to increase the fluidity of the lipid portions of stratum corneum, and thereby enhances the permeability of the skin layers for drug molecules (Golden et al., 1987 a). Isopropyl alcohol causes only some lipid extraction but no fluidization of the intercellular domains (Guy et al. 1989).

A very interesting result was revealed by the permeation rate of the diluted formulation of Soventol cream/WHS 1:2. Fig. 4.34. shows the considerable reduction of hydrocortisone permeation flux which was found to be about five times lower than that of Soventol 1% (Table 4.28.) (the permeation coefficient is relatively high because the starting concentration is reduced to the third). Considering the fact that hydrocortisone is suspended in the base these results strongly suggest that the reduced permeation profile of hydrocortisone from Soventol cream upon dilution is probably due to the reduced concentration of the enhancer and not that of the drug.

This finding allows the conclusion that dilution of cream bases could indeed alter drug permeation depending on the vehicle composition in a manner which could not be predictable, i.e. the 1:2 dilution of Soventol cream resulted in a 1:5 reduction in drug permeation.

Summarized discussion of the permeation experiments

The permeation profiles of hydrocortisone from different cream bases through excised human stratum corneum were found to differ insignificantly. The type of the base whether hydrophilic or lipophilic, water content, viscosity and the degree of saturation of the drug in the vehicle seem to play no role in drug permeation through stratum corneum. For all the preparations being suspension vehicles the variations in drug solubility in the formulation through changing the vehicle is always compensated by the reverse change in the partition coefficient of the drug between vehicle and stratum corneum, thus having no influence on drug permeation through stratum corneum. From equation 4.5. it was evident that the factor affecting permeation is the saturation concentration of the drug in the stratum corneum which is drug dependent.

Diluting 1% WHS with the other bases in the ratios 1:1 or 1:3 did not influence hydrocortisone permeation revealing the negligible effect of dilution on drug permeation. It should be noted that the diluted bases are still suspension vehicles, thus the two factors which were altered through dilution, the vehicle composition and the amount of the drug suspended in the vehicle, have no influence on permeation as mentioned above.

However, if the vehicle contains a permeation enhancer, which is capable of causing change in the barrier structure of the stratum corneum, the permeation of the drug through the stratum corneum is significantly promoted. Soventol cream which contains isopropyl myristate as well as isopropyl alcohol as enhancers showed a remarkable increase in hydrocortisone permeation compared with WHS 1%. Moreover, diluting Soventol cream with WHS in the ratio 1:2 caused a great decrease in permeation profile of hydrocortisone due to reduced enhancer concentration.
Briefly, it could be noted that, any suspension-type vehicle exerts its effect, independent of the base used and of the drug concentration beyond saturation provided that there is enough substance to guarantee a constant flux for application interval; drug liberation is fast enough and there are no specific effects on the skin barrier (Malzfeldt et al., 1989).

4.5. Influence of vehicle on structure of stratum corneum

Stratum corneum, the outermost layer of the skin, provides an outstanding barrier against the external environment and is also responsible for skin impermeability towards most solutes. The barrier function is related to the unique composition of the stratum corneum lipids and their complex structural arrangement. Alteration in the lipoidal matrix of the stratum corneum, therefore, increases its permeability. A number of physical techniques are used to gain information about structure of stratum corneum. Some of these techniques are differential thermal analysis, wide angle and small angle x-ray diffraction and infra-red spectroscopy (Bouwstra et al., 1991). In the following chapter differential scanning calorimetry as well as wide angle x-ray diffraction were used to investigate the interactions between vehicles used for permeation and structure of stratum corneum, which could provide a further explanation for the results obtained in the permeation experiments.

4.5.1. Differential scanning calorimetry of stratum corneum

4.5.1.1. Differential scanning calorimetry of stratum corneum pretreated with WHS, WWS, WS, NHC and HS

Isolated sheets of human stratum corneum show four characteristic endothermic transitions at about 40, 75, 85 and 105°C respectively (Van Duzee, 1975; Golden et al., 1987 b; Leopold et al., 1995). The first three transitions are lipid-based since they disappear after extraction of the samples with organic solvents.

The first transition is generally small and its occurrence varies from sample to sample. Until recently it has been attributed to the melting of sebaceous lipids (Golden et al., 1986). Today it is thought to represent a transition from an orthorhombic (crystalline) to a hexagonal lipid (gel state) subcell arrangement within the lipid bilayers (Bouwstra et al., 1992). The two thermal transitions at 75 and 85°C have been reported to represent phase transitions of the lipid bilayers from the lamellar gel state to the liquid crystalline state (Bouwstra et al., 1991). The transition at 85°C is considered by several investigators to represent a phase transition of lipids which are associated with proteins (Golden et al., 1987 b; Barry et al., 1991;

Bouwstra et al., 1991). The fourth transition at 105°C represents the denaturation of the protein portion of the stratum corneum, i.e. the α proteins (Van Duzee, 1975). The enthalpy of this transition is very sensitive for the water content in the stratum corneum. At lower water contents (less than approximately 15% w/w) this transition completely disappears (Bouwstra et al., 1991).

Generally, in order to obtain more distinct endothermic transitions the stratum corneum sheets should be hydrated to about 20% w/w (Van Duzee, 1975). Therefore, the stratum corneum sheets were incubated at room temperature for 48 hrs in a closed chamber of 75% relative humidity.

It has to be mentioned that the first peak, which is not easily detectable, as well as the α protein denaturation peak, which requires a certain water content of the sample in order to become apparent, could not be detected. Therefore, all DSC curves were evaluated with regard to the peak maximum temperatures of the distinct lipid-phase transitions at about 70 and 80°C. These two transitions give the required information about any possible interaction between the vehicle and the lipoidal structure of the stratum corneum, which is responsible of its barrier function.

The isolated human stratum corneum sheets were pretreated with the vehicles for 30 minutes at 37°C and the experiment was performed as described in 3.2.2. Typical DSC thermograms of untreated stratum corneum as well as stratum corneum pretreated with the various chosen vehicles containing 1% hydrocortisone are shown in Fig. 4.35.

Fig. 4.35. and Table 4.29. show that none of the tested bases produced significant changes in the lipid-phase transitions of the stratum corneum, as the maximum peak shift observed was 1.6°C which is considered to be insignificant. In conclusion, the vehicles investigated do not interact with stratum corneum structure; therefore, the barrier properties and subsequently the permeability of the stratum corneum remain unchanged. These results explain why all tested vehicles showed similar permeation profiles.

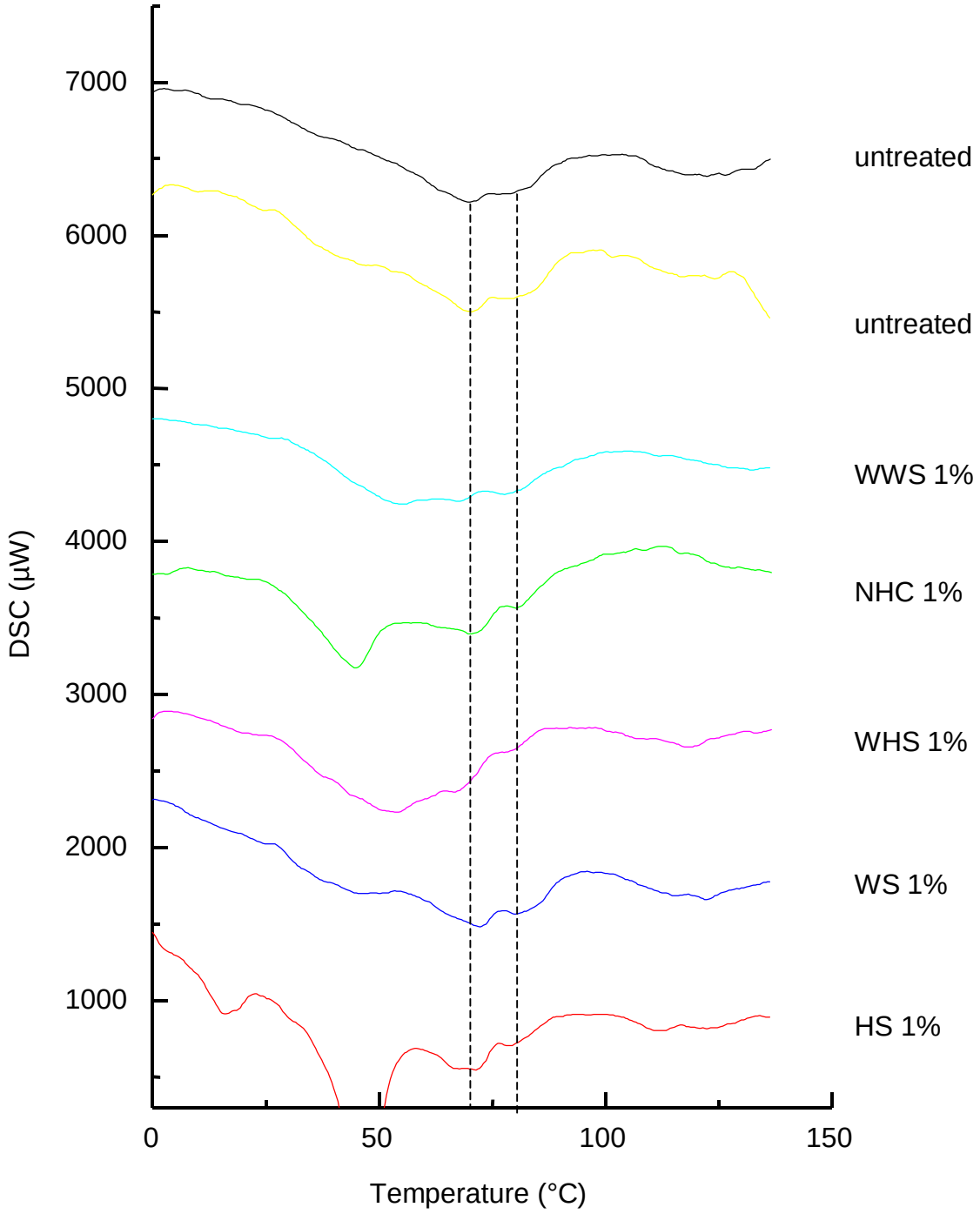
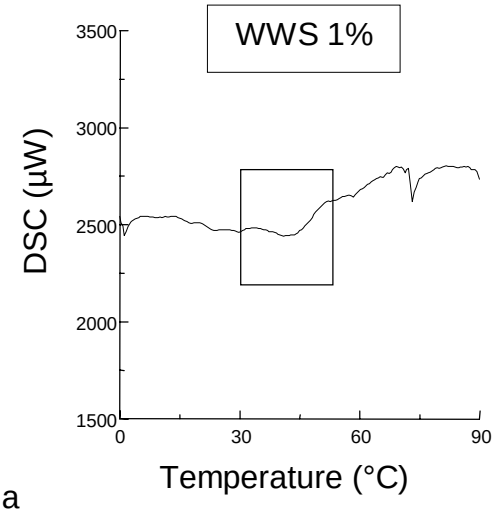


Fig. 4.35. DSC thermogram for untreated and vehicle (1% hydrocortisone) pretreated stratum corneum. Donor: Male, abdomen, 26 years

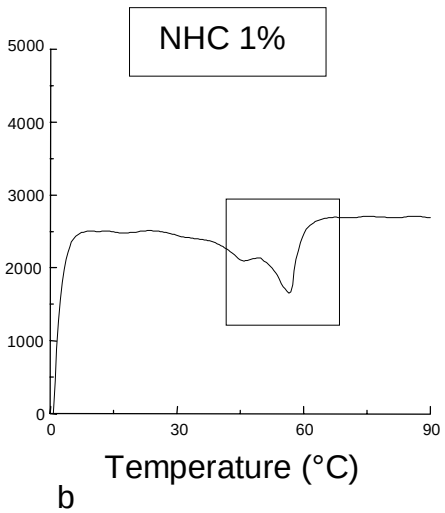
Stratum corneum	Peak 1 (C°)	Peak 2 (°C)
untreated	69.9	80.4
WWS 1%	68.3	79.8
NHC 1%	70.5	80.4
WHS 1%	68.8	80.4
WS 1%	71.4	80.7
HS 1%	70.7	80.1

Table 4.29. Peak maximum temperatures of the second and third phase transitions of untreated and 1% vehicle pretreated stratum corneum. Donor: Male, abdomen, 26 years

The peaks detected at temperatures below 70°C were considered to be produced from the base rests which remain adhering to the stratum corneum after pretreatment. To ensure this, all vehicles were also measured separately. Fig 4.36a, b, c, d and e reveal peaks characteristic to the bases at temperatures below 70°C for WWS, NHC, WHS, WS and HS respectively.



a



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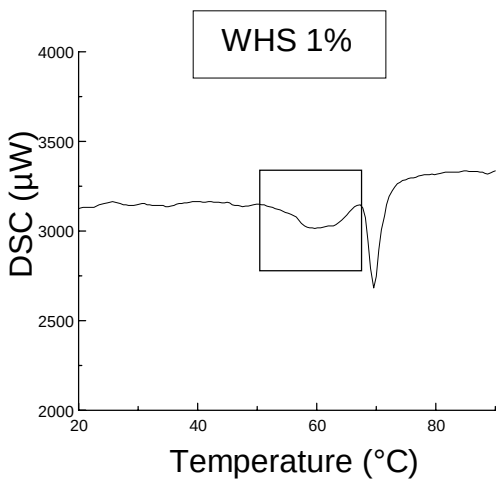

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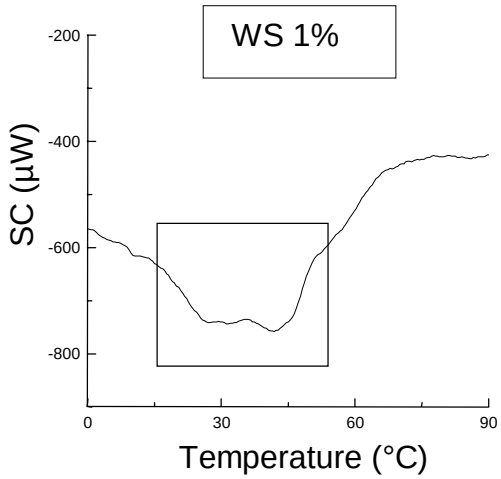

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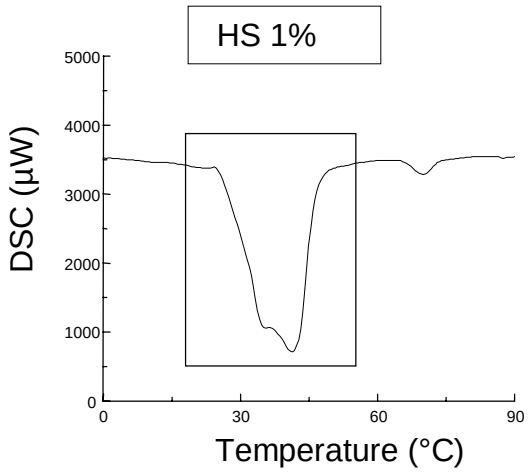

e

Fig. 4.36a, b, c, d and e DSC examination of 1% WWS, NHC, WHS, WS and HS respectively

To prove the absence of any interferrence due to the presence of hydrocortisone in the bases, the DSC interaction experiment was repeated using stratum corneum pretreated with hydrocortisone free vehicles. It is to be noted that identical results like those shown in Fig. 4.35. and Table 4.29. were obtained proving thereby that hydrocortisone does not influence the DSC curves exhibited by the vehicles.

Testing the 1:1 diluted vehicles for their interaction with stratum corneum seemed to be not important, as it is expected to have no influence on stratum corneum structure since the pure bases did not show any effect.

4.5.1.2. Differential scanning calorimetry of stratum corneum preatreated with Soventol cream, isopropyl myristate and isopropyl alcohol

The permeation rate of Soventol cream with 1% hydrocortisone was significantly high compared with that of WHS 1% (Fig. 4.34.). In order to explain this finding the interactions between Soventol cream and stratum corneum were investigated via DSC.

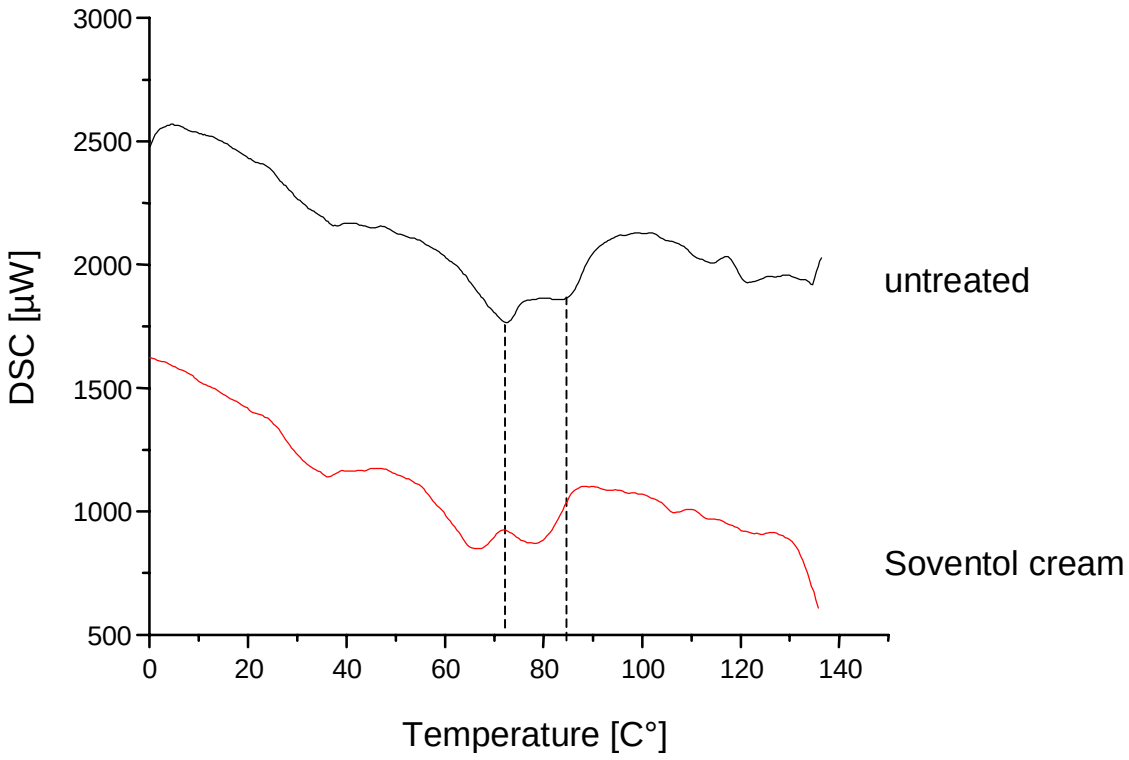

Fig. 4.37. DSC thermogram for human stratum corneum after pretreatment with 1% Soventol cream. Donor: Male, breast, 26 years

The pretreatment of human stratum corneum with 1% Soventol cream revealed a significant shift of about 5°C to lower temperatures in both peaks (Fig. 4.37. and Table 4.30.). This shift indicates an alteration in the lipoidal structure of stratum corneum which is known to be responsible for its barrier properties subsequently leading to an increase in its permeability. This result, therefore, explains the remarkable high permeation rate of hydrocortisone from Soventol cream compared to

that of WHS which was found to have no interaction with stratum corneum lipids (Fig. 4.35.).

Stratum corneum	Peak 1 (C°)	Peak 2 (C°)
Untreated	72.68	84.53
Pretreated with Soventol cream	66.86	78.97

Table 4.30. Peak maximum temperatures of the second and third phase transitions of untreated and soventol cream pretreated stratum corneum. Donor: Male, breast, 26 years

This result is believed to be referred mainly to the presence of isopropyl myristate which is known to possess a pronounced penetration enhancing effect (Fang, 1996). To investigate whether the observed effect is indeed caused by isopropyl myristate an interaction experiment between stratum corneum and isopropyl myristate was performed. As expected similar alteration of the phase transition temperatures of the stratum corneum lipids was observed upon treatment with isopropyl myristate (Fig. 4.38 and Table 4.31.).

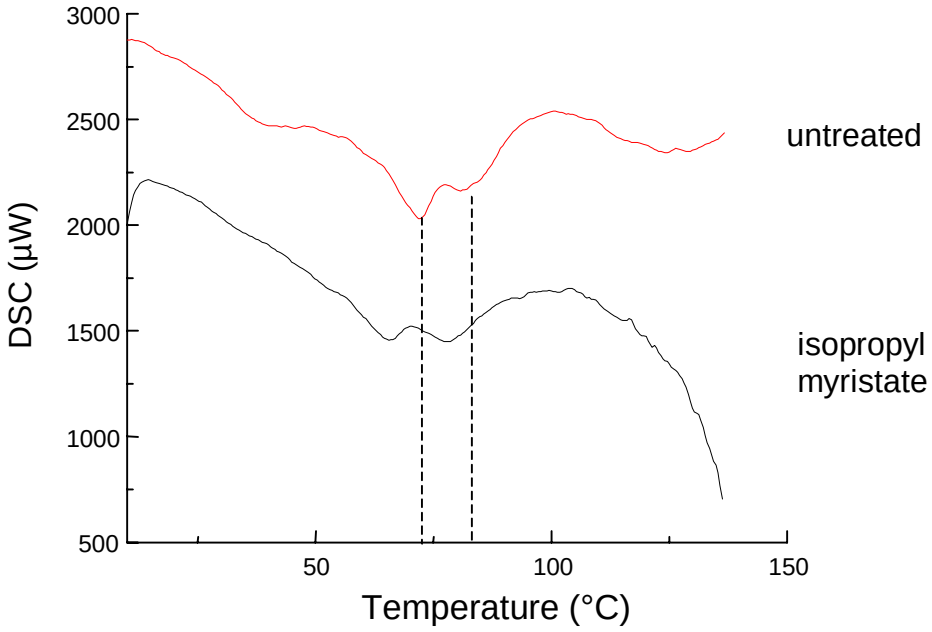

Fig. 4.38. DSC thermogram for human stratum corneum after pretreatment with isopropyl myristate. Donor: Male, breast, 26 years

Stratum corneum	Peak 1 (C°)	Peak 2 (C°)
Untreated	72.44	82.84
Pretreated with isopropyl myristate	65.71	77.94

Table 4.31. Peak maximum temperatures of the second and third phase transitions of untreated and isopropyl myristate pretreated stratum corneum. Donor: Male, breast, 26 years

This phenomenon may be interpreted as an increase in fluidity of the lipid bilayers; apparently interactions between the permeation-enhancing compound and the intercellular lipids lead to a less ordered state of the latter (Leopold et al., 1995).
This fluidizing action is interpreted either by the development of arrangements with periodic undulations (Rolland et al., 1991) or the formation of solid solutions (lamellar gel states consisting of a homogeneous mixture of the lipids and the vehicle molecules).
The reason for the fluidizing effect of isopropyl myristate might be its branched structure (Leopold et al., 1995). Isopropyl myristate is believed to operate similar to oleic acid by penetrating into the lipid structure with its polar end close to the lipid polar heads (Smith and Maibach, 1995) (Fig. 4.39.). Because of its branched structure, it then disrupts the packing of the intercellular lipids and increases their fluidity. Drug mobility in this less tightly packed arrangement will then increase (Barry, 1987 a).

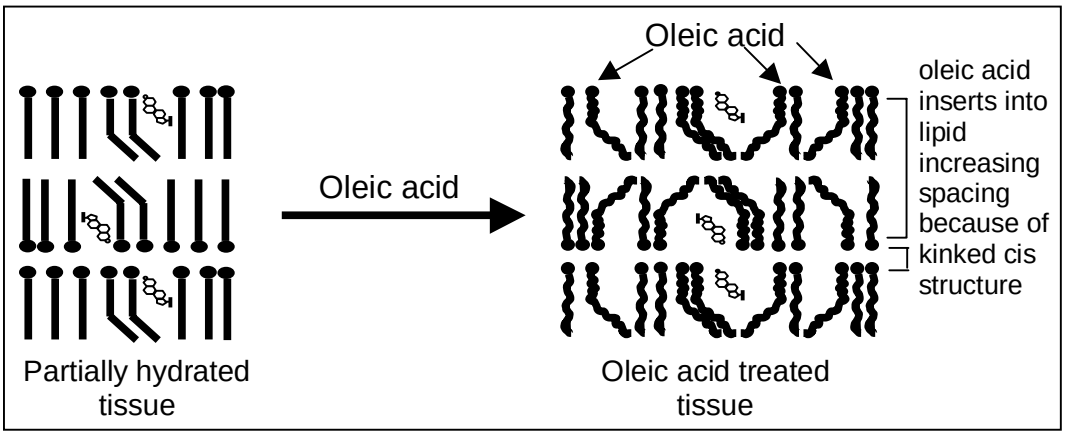

Fig. 4.39. Scheme illustrating the mode of action of oleic acid as penetration enhancer (Barry, 1987 a).

In order to reveal the role of isopropyl alcohol in enhancing hydrocortisone permeation, a similar interaction experiment was carried out.

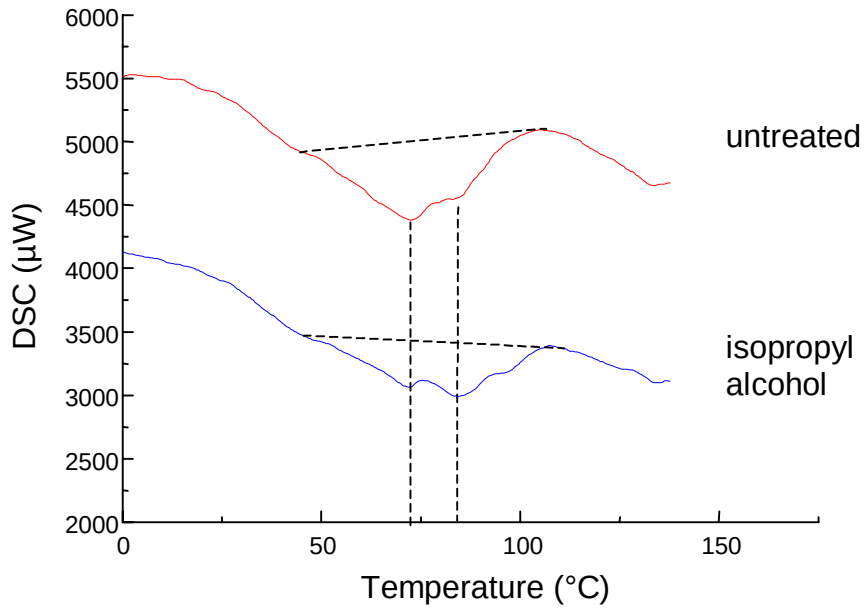

Fig. 4.40. DSC thermogram for human stratum corneum after pretreatment with isopropyl alcohol. Donor: Female, breast, 57 years

Stratum corneum	Peak 1 (C°)	Peak 2 (C°)	Enthalpy of both peaks in a temperature range of ~ 47-105°C (mJ/mg)
Untreated	73.09	84.42	18.0
Pretreated with isopropyl alcohol	72.67	84.42	15.8

Table 4.32. Peak maximum temperatures of the second and third phase transitions and the enthalpy of both peaks of untreated and isopropyl alcohol pretreated stratum corneum. Donor: Female, breast, 57 years

Isopropyl alcohol is known to act mainly via extraction of stratum corneum lipids, therefore the DSC curves were evaluated with regard to the peak maximum temperatures of the lipid-phase transitions as well as the phase transition enthalpies. Fig. 4.40. and Table 4.32. show that the pretreatment of stratum corneum with isopropyl alcohol did not reveal a significant alteration in the phase transition

temperatures. Moreover, the effect observed in the phase transition enthalpies (~12.2%) was considered to be also insignificant (Leopold and Lippold, 1995 reported a statistically significant effect for an enthalpy change of more than 17%). This finding proves that isopropyl myristate is alone responsible for the great permeation profile of hydrocortisone from Soventol cream.

4.5.2. Wide- angle X-ray diffraction (WAXD) of human stratum corneum

Wide-angle X-ray experiments are used to investigate the molecular organization of barrier components of human stratum corneum. It gives information about the lipid packing arrangements in the intercellular bilayers as well as the protein structure in the intracellular keratin. WAXD experiments were performed to detect the interactions between the vehicles used for permeation and stratum corneum structure in order to confirm thereby the results obtained in the DSC-experiments.

In the untreated stratum corneum two characteristic sharp and intense reflections at 0.367 and 0.409 nm were observed (Table 4.33.). The diffraction ring at 0.367 corresponds to an orthorhombic crystalline structure, while the reflection at 0.409 is probably due to hexagonal packing of the alkyl chains (White et al., 1988).

Normally, two diffuse diffraction rings at 0.46 nm and 0.98 nm are detected in human stratum corneum. The reflection at 0.46 nm is suggested to be due both to hydrocarbon chains in the liquid state and to soft keratin located in the corneocytes, whereas the diffraction ring at 0.98 is only attributed to soft keratin (Bouwstra et al., 1992). These two reflections are not detected in the present work.

Since the reflections at 0.367 and 0.409 nm become sharper upon hydration of stratum corneum from 6-20% (Bouwstra et al., 1992) the stratum corneum sheets were incubated for 48 hrs in a closed chamber of 75% humidity to obtain a water content of 20% w/w. The wide angle X-ray diffraction experiments were performed as described in 3.2.3.

4.5.2.1. Wide- angle X-ray diffraction (WAXD) of human stratum corneum of WHS, WWS, WS, HS and NHC

Table 4.33. shows the lack of interaction between all tested vehicles and the lipoidal structure of stratum corneum as the two reflections at 0.367 and 0.409 nm remained unchanged after pretreatment. In conclusion, no change in the permeability of

stratum corneum for hydrocortisone should be expected using different vehicles for permeation. This result is in agreement with that obtained in the differential scanning calorimetry study.

Stratum corneum pretreated with	Diffraction ring [nm]	
-	0.367	0.409
WHS 1%	0.367	0.409
WS 1%	0.367	0.409
WWS 1%	0.367	0.409
NHC 1%	0.367	0.409
HS 1%	0.367	0.409

Table 4.33. Wide-angle X-ray diffraction of untreated and with 1% WHS, WS, WWS, NHC and HS pretreated stratum corneum. Donor: Male, abdomen, 26 years

4.5.2.2. Wide-angle X-ray diffraction (WAXD) of human stratum corneum pretreated with 1%Soventol cream

Treating the stratum corneum with soventol cream which is known to contain a permeation enhancer, isopropyl myristate, induced an obvious shift of the two reflections to higher values (Table 4.34.). This shift indicates an alteration in the intercellular lipid domains of stratum corneum leading to its increased permeability towards permeation substances.

Stratum corneum pretreated with	Diffraction ring [nm]	
-	0.367	0.409
Soventol cream	0.371	0.413

Table 4.34. Wide-angle X-ray diffraction of untreated and with Soventol cream pretreated stratum corneum. Donor: Male, abdomen, 26 years

WAXD experiments for stratum corneum pretreated with isopropyl myristate and isopropyl alcohol were not carried out because there was no more stratum corneum of the same donor available. Their effect is expected to match with the DSC investigations like all previous results.

4.5.3. Summarised discussion of differential scanning calorimetry and wide-angle X-ray diffraction experiments and their correlation with the permeation study

The vehicles WHS, WWS, NHC, HS and WS containing 1% hydrocortisone neither produce significant change in the peak maximum temperatures of the distinct lipid phase transitions at about 70 and 80°C nor change the reflections of the intercellular lipid domains of the stratum corneum. These findings obtained from the differential scanning calorimetry study as well as the wide angle X-ray diffraction experiments respectively, allow the conclusion that the components of these vehicles (the lipophilic phase, hydrophilic phase, water content and surfactants) do not interact with the lipoidal structure of the stratum corneum thus having no influence on its barrier properties. Subsequently, its permeability towards permeating substances remains unchanged. This finding adds a further explanation to the results obtained in the permeation experiments as the permeation profiles of hydrocortisone from all investigated vehicles through stratum corneum were found to differ insignificantly.

Nevertheless, pretreating stratum corneum with Soventol cream revealed a shift of the peak maximum temperatures of the second and third phase transitions of the stratum corneum of about 5°C to lower temperatures. Moreover, the wide-angle X-ray diffraction study showed a shift in both reflections at 0.367 and 0.409 nm to higher values. Both shifts indicate the disruption of the packing of the intercellular lipids of the stratum corneum.

This effect was thought to be attributed to the presence of isopropyl myristate which is known to act as a penetration enhancer. Its effect on stratum corneum structure was investigated using differential scanning calorimetry technique. It is to be noted that similar shifts of the second and third phase transitions to lower temperatures were observed. Isopropyl myristate is believed to penetrate into the lipid structure of stratum corneum with its polar end close to the lipid polar heads increasing thereby the fluidity of the intercellular lipids.

This result interprets the significant high permeation rate of 1% Soventol cream over that of WHS 1% which was proven to cause no alteration in the structure of stratum corneum. Taken altogether, these results suggest that the great reduction in the permeation profile of hydrocortisone from the diluted formulation (Soventol cream/WHS 1:2) is attributed to reduced enhancer concentration and not to the decrease in hydrocortisone concentration.

5. Conclusion

The influence of diluting semisolid preparations on drug release and permeation through excised human stratum corneum was investigated. For this purpose the model cream, water containing hydrophilic ointment DAB 1998 (WHS) with 1% hydrocortisone was diluted with various hydrophilic and lipophilic bases chosen from the German Pharmacopoeia 1998. Moreover, the different factors affecting hydrocortisone liberation and permeation were investigated in order to be able to interpret the different liberation and permeation profiles.

5.1. Drug release experiments

WHS as well as the other cream bases, NHC, WS, WWS and HS were prepared with 1% hydrocortisone and tested for drug liberation in order to reveal the influence of the base type whether lipophilic, hydrophilic, hydrous or anhydrous on drug release. It was evident that o/w systems (WHS, NHC) revealed very high release rates in contrast to the anhydrous hydrophilic and lipophilic bases (WS, HS) and w/o system (WWS) which showed remarkably low liberation profiles. This finding was referred to the fast and free release of hydrocortisone from the external aqueous phase of the o/w vehicles in addition to the relatively high solubility of hydrocortisone in these bases. The great influence of water content observed in the hydrophilic bases on drug release and solubility (WHS, HS) was not noticed in the case of the lipophilic vehicles, as WS and WWS showed almost identical liberation rates associated with very low solubility of hydrocortisone. The slightly greater release of hydrocortisone from HS over WS being both anhydrous bases is considered to be due to the increased solubility of the drug in the former which is probably due to the solubilizing effect of the emulsifying alcohols.

To study the effect of dilution on drug liberation, 1% WHS was diluted with the same base, WHS, and the other bases in the ratios 1:1, 1:2 and 1:3.
For the facts that WHS and NHC possess similar microstructure, identical dissolving capacity and release rate for hydrocortisone the diluted formulations of both bases

revealed, as expected, similar liberation profiles. The relatively high release rates of the preparations were attributed to the o/w character of the bases and the significantly high saturation concentration of hydrocortisone. However, it is to be noted that the liberation rates were higher than predicted for all mixing ratios. Since all bases are suspension vehicles having identical amount of dissolved hydrocortisone the factor influencing drug release in this case was considered to be the variation in concentration gradient between the suspended and dissolved drug upon dilution.

The dilution of WHS 1% with WWS 1:1 resulted in a quite high liberation rate if compared with the dilutions 1:2 and 1:3. Conductivity and colouring tests showed that this combination is still an o/w system, whereas increasing the amount of WWS in the mixture converts it into a w/o one. However, this combination was not accompanied by a respective increase in the drug solubility indicating that the influence of base type being o/w exceeds in this case the effect of drug solubility on hydrocortisone release.

The great decrease in liberation of hydrocortisone, that was noticed upon dilution of WHS 1% with HS at the ratio 1:1, despite of being still an o/w system, confirms that hydrocortisone is dissolved to a great extent in the external aqueous phase. Decreasing the amount of aqueous phase by that dilution therefore affected the drug release negatively. When the amount of water was further decreased in the dilution ratios 1:2 and 1:3 a phase conversion has taken place, which was accompanied by a pronounced reduction in drug liberation.

Diluting WHS 1% with WS resulted in all combinations in very low liberation rates with no significant difference, which indicates, that a phase conversion has taken place already with the dilution ratio of 1:1. For the same reason the release of hydrocortisone was drastically reduced when WHS 1% was diluted 1:1 with the lipophilic vehicle, white petrolatum.

A comparative study on the formulations WHS/HS 1:1 and WHS/WS 1:1 with WHS/white petrolatum 1:1 was undertaken to reveal the influence of adding a hydrophilic and lipophilic emulsifier to white petrolatum, respectively. It was found that the hydrophilic emulsifier in HS retained the o/w character of the base for that water content (35%) besides, it increased the solubility of the drug in the base, resulting in having the greatest liberation profile among the three formulations.

Furthermore, it was noticed that diluting WWS 1% with WHS 1:1 showed exactly the same liberation profile as the formulation WHS 1%/WWS 1:1. The same phenomenon was observed for the formulations NHC 1%/WHS 1:1 and WHS 1%/NHC 1:1. In conclusion, incorporating the drug in either of the two bases before dilution does not affect drug release from the final formulation.

Moreover, WHS was prepared with different amounts of water in order to investigate the effect of water content in hydrophilic bases on hydrocortisone release. Results showed that the water content greatly influences drug release as long as the system is an o/w one. However, very low water contents resulted in phase conversion after which any variations in the amount of aqueous phase did not influence drug release. In WHS the phase conversion took place between 30 and 35% water.

From the above-mentioned observation it seems surprising that NHC having only 50% water content releases hydrocortisone identical to WHS which contains 70% water. This effect was considered to arise from differences in the type and amount of emulsifier. Therefore, both bases with equal amounts of emulsifier (9%) and water (50%) were tested for hydrocortisone release. Significant greater drug liberation and solubility were noticed for NHC (9%) indicating thereby the greater influence of the non-ionic surfactant in NHC over the anionic one in WHS on drug release and solubility. However, comparing the release of NHC (9%) and NHC (15%) showed no difference revealing the negligible effect of surfactant concentration. Furthermore, the increased hydrocortisone release and solubility of NHC anhydrous over HS confirm the effect of the emulsifier type on hydrocortisone solubility and release away from any influence of the aqueous phase. Moreover, the fact that NHC anhydrous possesses identical dissolving capacity for hydrocortisone to that of NHC (50% water) indicates that water does not affect the drug solubility in bases emulsified by non-ionic emulsifiers as does in vehicles containing ionic ones. This finding suggests that the drug is present mainly in the structure of the base and not in the aqueous phase unlike the case of WHS, besides it explains why NHC and WHS have identical release rates despite of different water contents.

5.2. Rheological experiments

In addition to base type, water content and drug solubility, the viscosity of the bases was found to add further explanations for the different drug releasing profiles. Oscillatory measurements were carried out to investigate the bases in their viscoelastic range. In this range the structure of the base is undisturbed. The bases were compared with regard to the complex viscosity, phase angle, storage modulus and loss modulus.

For all the vehicles, 1% WHS, WWS, HS, WS and NHC the phase angle was below 45° indicating exceeding elastic behaviour. Water content was found to have no influence on viscosity when incorporated inside the base as WS and WWS revealed almost identical viscosities. In contrast, when water content constituted the external phase it influenced the viscosity greatly as expressed in the significantly greater viscosity of HS over WHS. These findings match with the releasing profiles of these bases. Nevertheless, the well known rule that a decrease in viscosity is accompanied by an increase in drug release was only partly verified. The lipophilic bases WWS and WS in spite of having much lower viscosities than HS, they possessed lower release rates for hydrocortisone. In this case the effect of base type and drug solubility on drug release probably dominated over the effect of viscosity. Furthermore, the greater viscosity of NHC over WHS, which is probably due to lower water content in the former, does not agree with their identical liberation profiles. The increase in viscosity was thought to compensate the drug release promoting effect of the non-ionic emulsifier in NHC resulting in a release identical to that of WHS.

The significant increase in the phase angle - indicating increased viscous behaviour - observed for all dilution ratios of WHS/WS and the combinations 1:2 and 1:3 of WHS with both HS and WWS, was probably a result of the great structural change associated with the phase conversion of all these vehicles from the o/w system to the w/o one. Regarding the values of the storage and loss modulus however shows that there is a great increase in the values of both, which means that the elasticity of the vehicles greatly increased, too. This was accompanied by a great increase in viscosity adding thereby a further explanation for the enormous drop in drug liberation noticed for these formulations.

It is worthy to note that, in contrast, all hydrophilic (o/w) bases – all dilution ratios of WHS/NHC and the 1:1 combination of WHS/HS and WHS/WWS- revealed an insignificant change in the phase angle. This does not mean that the viscous and elastic behaviour of these vehicles remained constant as the values of both moduli also increased. The increase of their values was however in a balanced manner so that the ratio between viscous and elastic properties remained almost constant.

For these bases being o/w systems their viscosities are greatly affected as shown above by the water content unlike the w/o preparations. This finding explains the greater viscosity of WHS/HS 1:1 having 35% aqueous phase over that of WHS/WWS 1:1 with 60% water content. This is probably the reason why the latter had a significant greater release rate than the former despite of its lower solubility for hydrocortisone.

Nevertheless, the significant greater solubility of hydrocortisone in WHS/NHC 1:1 over WHS/WWS 1:1 is probably the reason behind its greater drug release despite of its greater viscosity, which is completely opposing the case mentioned above.

Reducing the amount of the non-ionic emulsifier in NHC from 15 – 9% did not alter the drug solubility and release, as shown before, which was therefore interpreted as the negligible influence of this change in surfactant concentration on hydrocortisone liberation. Surprisingly the decreased amount of surfactant, however, resulted in a significant decrease in viscosity, which would let us rather expect an increase in drug release. These results strongly suggest that the emulsifier not only improves the drug solubility, but also plays a role in facilitating the drug release. Therefore, the decrease in surfactant concentration in NHC (9%), i.e. decrease in the drug release promoting effect, was probably compensated by the decrease in viscosity resulting in identical drug release to that of NHC (15%).

Taken altogether the drug release is affected by a number of factors. These factors can be summarized as follows:

a) Factors promoting drug release: type of base being o/w, increased drug solubility, increased water content in o/w bases and decreased viscosity.

b) Factors limiting drug release: type of base being w/o, decreased drug solubility, decreased water content in o/w bases and increased viscosity.

These facts are true for most cases, however sometimes not all factors affect the drug release in one direction i.e. some factors promote drug liberation whereas other

factors limit it. In this case the effect of one of the factors either exceeds the effect of the others or the opposing factors are equally strong so that they compensate each other. This can not be expected in advance and is greatly case-dependent.

5.3. Permeation experiments

Permeation experiments were carried out in order to investigate the influence of the different base types and their dilutions on drug permeation. The permeation of hydrocortisone was tested through excised human stratum corneum, which presents the main barrier of the skin. To be able to correlate the permeation experiments to the release experiments the same vehicles were used.

All vehicles were found to have similar permeation profiles for hydrocortisone i.e. all the factors affecting drug release like base type, water content, drug solubility and viscosity of the base had no influence on drug permeation through excised stratum corneum. The lack of correlation between release and permeation experiments is also reported by many authors (Lippold, 1984; Zatz et al., 1996; Alberg, 1998). On the other hand, this result may not agree with other studies (Shah et al., 1991) which report a greater drug permeation for ointments. However, this increased drug permeation observed for ointments is due to their occlusive effect which can unfortunately not be detected by this in vitro method. Equation 4.5 states that in case of suspension vehicles the factor directly influencing drug permeation is the saturation concentration of the drug in stratum corneum not that in the vehicle, therefore different base types with different drug solubilizing capacities do not affect drug permeation. Consequently, all the 1:1 and 1:3 diluted vehicles revealed very close permeation profiles with no significant differences. Furthermore, the permeation rates of the diluted formulations lay in the same range of that of WHS 1%, revealing thereby the negligible influence of dilution on hydrocortisone permeation. This was attributed to the fact that all diluted bases are still suspension vehicles i.e. dilution does not reduce drug concentration below its degree of saturation. For this the concentration of hydrocortisone in stratum corneum remains unchanged, subsequently its permeation.

In contrast, hydrocortisone was found to permeate significantly greater from Soventol cream than from WHS. This was believed to be due to the enhancing effect of isopropyl myristate which is present in Soventol cream. For all the above-mentioned

findings the considerable reduction of hydrocortisone permeation following the dilution of Soventol cream 1:2 with WHS is believed to be due to reduced enhancer concentration and not that of the drug.

The findings obtained in the present study substantiated by the respective literatures allow us to conclude that dilution may affect drug permeation through stratum corneum in the following cases:

1- Changing the occlusive properties of the base by diluting it with a different base type.
2- The presence of an enhancer in either of the 2 bases (the diluent or/and the original base)
3- When the drug is completely dissolved in the base, or in case of diluting a suspension vehicle below its degree of saturation, the drug solubility in stratum corneum may be affected upon dilution.

5.4. Influence of vehicle on structure of stratum corneum

In the present study the interactions between vehicle and stratum corneum were investigated using differential scanning calorimetry and wide angle x-ray diffractometry. It is well known that the lipoidal structure of stratum corneum is responsible for its barrier properties (Golden et al., 1987 a). Hence, an alteration in the lipoidal structure would indicate an increase in the permeability of the barrier towards invasive substances. The lack of interaction between the components of WHS, WS, WWS, HS and NHC and the lipid bilayers of stratum corneum proven by both techniques therefore explains why all these vehicles showed similar permeation profiles for hydrocortisone.
On the other hand, a significant shift of the lipid phase transitions of stratum corneum was observed upon pretreating the stratum corneum with Soventol cream. This was also confirmed by the WAXD technique showing an analogous shift in the diffraction rings characteristic for the intercellular lipid domains. These findings indicate the disruption of the packing of the intercellular lipids of stratum corneum. Isopropyl myristate, which is known to act as a penetration enhancer, was proven to be responsible for these effects as it exhibited similar shifts of the phase transition

temperatures. This phenomenon may be interpreted as an increase in the fluidity of the lipid bilayers. The reason for the fluidizing effect of isopropyl myristate might be its branched structure, which may cause a decrease of the ordered structure of the lipid lamellae. Moreover, isopropyl myristate is able to dissolve considerable amounts of cholesterol (Leopold, 1992), which may act as a membrane stabilizer (Imokawa, et al., 1989). In conclusion, the increased permeability of hydrocortisone from Soventol cream in comparison to WHS is attributed to the increased disorder of the stratum corneum structure caused by isopropyl myristate.

6. Summary

Dilution of semisolid preparations, in order to tailor the formulations to the needs of the patients, was thought to be associated with a number of dangers, one of which is the unpredictable reduction of activity. In the present study the influence of dilution on hydrocortisone liberation and permeation through excised human stratum corneum was investigated. Furthermore, the factors affecting both processes and their correlation were examined. Water containing hydrophilic ointment DAB (1998) with 1% hydrocortisone was taken as a model cream; it was diluted with various bases possessing different compositions and properties.

The type of the base, was found to be one of the most important factors affecting drug release. The liberation profiles of hydrocortisone from o/w systems were significantly greater than from anhydrous bases and lipophilic vehicles. This was believed to be due to free and fast release of the drug from the external aqueous phase. Moreover, for all the investigated vehicles being suspension-type, the degree of hydrocortisone solubility had also a direct influence on drug release. Almost all vehicles showing high liberation rates exhibited relatively high saturation concentrations of hydrocortisone. An additional factor playing a direct role in drug release is the rheological behaviour of the bases. The investigations were carried out using oscillatory measurements, which examine the vehicles in their linear viscoelastic range. The o/w systems were characterized by having relatively low viscosities, which add a further explanation for their great liberation profiles. Diluting the o/w base with lipophilic or anhydrous hydrophilic bases resulted in a phase conversion to the w/o system. This was associated with a great increase in viscosity and very low liberation rates. Furthermore, water content when present in o/w systems revealed a significant influence on drug liberation. The increased water content raised in most cases the amount of dissolved drug and decreased the viscosity; both resulted in increased hydrocortisone liberation. Moreover, it was found that the non-ionic emulsifiers in o/w vehicles promoted drug release over the anionic ones.

The results obtained from the permeation studies revealed the lack of correlation between liberation and permeation studies, i.e. all factors which were found to affect hydrocortisone release like base type, drug solubility and viscosity had no influence on drug permeation resulting in no significant differences in the drug permeation from the various cream bases studied. In conclusion, the permeability of stratum corneum, which was not affected by the cream bases as shown by DSC and WAXD experiments, is the rate limiting step for drug permeation. Furthermore, the dilution did not result in any reduction in drug permeation, which was referred to the fact that all diluted bases are still suspension vehicles. In contrast to WHS, hydrocortisone was found to permeate significantly greater from Soventol cream (placebo with 1% hydrocortisone), which is known to contain isopropyl myristate as permeation enhancer. DSC experiments showed that the pretreatment of human stratum corneum with 1% Soventol cream as well as with isopropyl myristate revealed a significant shift in the lipid phase transitions of about 5°C to lower temperatures. This shift indicates an alteration in the lipoidal structure of stratum corneum, which is known to be responsible for its barrier properties subsequently leading to an increase in permeability. Moreover, the 1:2 dilution of Soventol cream with WHS revealed a considerable reduction of hydrocortisone permeation.

In conclusion, dilution of suspension-type cream bases could indeed reduce drug permeation in the case they contain a penetration enhancer. However, this reduction in drug permeation could be in an unpredictable manner i.e. the 1:2 dilution of Soventol cream resulted in a 1:5 reduction in drug permeation. The reduced hydrocortisone permeation from the diluted formulation is believed to be due to reduced enhancer concentration.

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